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NATIONAL RESEARCH COUNCIL OF CANADA

PROCEEDINGS
OF THE
TWENTY-SEVENTH MEETING
OF THE
ASSOCIATE COMMITTEE ON
DENTAL RESEARCH

OTTAWA

FEBRUARY 23 - 24, 1961

ASSOCIATE COMMITTEE ON DENTAL RESEARCH

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February 23 - 25, 1961

Initial Distribution

The first session convened in the Council Chamber of the National Research Council, Ottawa, at 7:30 a.m., February 23, 1961.

I. Attendance:

Dr. H. J. Parviter, Chairman
 Eng. H. M. Deira
 Dr. L. F. Belanger
 Dr. H. Brown
 Dr. G. Cantaldi
 Dr. A. D'Arcy
 Dr. L. E. Francis
 Dr. A. G. Connell
 Dr. L. A. Milburn
 Dr. J. Kleinberg
 Dr. J. W. Ballantyne
 Dr. G. Stelfox
 Dr. A. I. Broder
 Dr. C. B. Scott
 Dr. R. J. Siller
 Miss D. J. Weigen, Secretary

By Invitation:

Dr. H. W. MacLean
 Dr. J. D. McLean

Regrets of their inability to attend were received from:

Dr. R. P. Farquharson
 Dr. C. F. Lobbed
 Dr. E. L. McE. H. Saunders
 Dr. G. L. Thomson

NATIONAL RESEARCH COUNCIL

Proceedings

The Chairman was a member of the Committee: Dr. A. J. Rhodes, Director of the School of Hygiene, University of Toronto, Dr. G. Nikiforuk, Division of Research, University of Toronto, and Dr. R. J. Slater, Research Institute, Hospital for Sick Children, Toronto. He also introduced the Twenty-Seventh Meeting, Dean Hector MacLean of the Faculty of Dentistry of the University of Alberta, and Dean James McLean of the Faculty of Dentistry of the University of Alberta.

ASSOCIATE COMMITTEE ON DENTAL RESEARCH

February 23 - 24, 1961

The first session convened in the Council Chamber of the National Research Council, Ottawa, at 9:30 a.m., February 23, 1961.

1. Attendance:

Dr. K. J. Paynter, Chairman

Brig. K. M. Baird

Dr. L. F. Belanger

Dr. H. Brown

Dr. C. Castaldi

Dr. A. D'Iorio

Dr. L. E. Francis

Dr. A. G. Gornall

Dr. L. A. Kilburn

Dr. I. Kleinberg

Dr. J. W. Neilson

Dr. G. Nikiforuk

Dr. A. J. Rhodes

Dr. G. D. Scott

Dr. R. J. Slater

Miss D. J. Wright, Secretary

By Invitation:

Dr. H. R. MacLean

Dr. J. D. McLean

Regrets at their inability to attend were received from:

Dr. R. F. Farquharson

Dr. C. P. Leblond

Dr. R. L. deC. H. Saunders

Dr. D. L. Thomson

The Chairman welcomed the new members of the Committee: Dr. A. J. Rhodes, Director of the School of Hygiene, University of Toronto, Dr. G. Nikiforuk, Division of Dental Research, University of Toronto, and Dr. R. J. Slater, Research Institute, Hospital for Sick Children, Toronto. He also introduced the two guests of the Committee, Dean Hector MacLean of the Faculty of Dentistry of the University of Alberta, and Dean James McLean of the Faculty of Dentistry of Dalhousie University.

2. Minutes of the Twenty-Sixth Meeting

The Minutes of the Twenty-Sixth Meeting had been distributed to all members and were therefore taken as read.

It was MOVED by Dr. Kleinberg and SECONDED by Dr. Scott

THAT the Minutes be approved.

CARRIED

3. Business Arising out of the Twenty-Sixth Meeting

(a) Cancellation of Fellowship (Proc. 26th Mtg., Min. 16)

A Fellowship in the amount of \$2,500 had been awarded to Mr. M. Wasylchuk to work in the Department of Biochemistry of the University of Alberta under the supervision of Dr. E. T. Pritchard. Mr. Wasylchuk was subsequently offered a Fellowship of greater value by the University of Minnesota and therefore declined the N. R. C. award

(b) Equipment Grant (Proc. 26th Mtg., Min. 17)

The Chairman noted that the Committee had considered, at its 26th Meeting, an application from Dr. J. P. Lussier for the sum of \$3,615 for the purchase of an electrometer and accessories. The application had been approved in principle but deferred for payment if funds became available.

Following Mr. Wasylchuk's decision not to accept the Fellowship offered to him, the amount of \$2,500 was awarded to Dr. Lussier as a contribution towards the cost of purchasing the electrometer.

(c) Release of Encumbrance (Proc. 26th Mtg., Min. 17)

At its 26th Meeting, the Committee had encumbered the amount of \$3,000 to be awarded as a grant to Dr. J. R. Trott of the University of Manitoba if he should submit a clear research proposal.

Dr. Trott subsequently submitted an application for a grant in aid of "A histochemical investigation into the glycogen content of the developing gingiva". This application was reviewed by the members of the Executive and a grant of \$3,000 was awarded in support of the project.

(d) Treatment of Experimental Animals (Proc. 26th Mtg., Min. 13)

The Chairman reported that he had forwarded to Dr. E.H. Bensley, Honorary Secretary of the Canadian Federation of Biological Societies, a recommendation that the Federation undertake the preparation of a code for the handling of experimental animals which might be put into effect in Canada.

Dr. Bensley later advised the Chairman that the Federation had created an ad hoc committee to submit recommendations to the Board of Directors of the Federation regarding a code of ethics for the treatment of experimental animals. The Chairman of this new committee is Dr. R. A. Waud, former Head of the Department of Pharmacology of the University of Western Ontario.

4. Chairman's Remarks

The Chairman outlined briefly the present status of dental research funds in Canada, the expansion of dental research in Canada and the future role of the Associate Committee on Dental Research.

(a) Present Status of Dental Research Funds in Canada

"Associate Committee on Dental Research: For the session 1960-61 the regular allocation to the Associate Committee on Dental Research of the National Research Council was \$91,000, but because of the large number of requests for funds which were received by the Committee, the President authorized the spending of an additional \$10,000, thus making the total allocation \$101,000. The standing commitments of the Dental Research Committee last year for term grants, continuing Fellowships, etc., were approximately \$46,000, which left a sum of about \$55,000 to be distributed among additional requests for \$120,000. This obviously necessitated difficult and drastic action on the part of the Committee, - action which unquestionably inflicted hardship in some cases.

"Because of this alarming shortage of funds to support dental research, the Committee submitted a budget request for the 1961-62 session of \$225,000, and the Executive was directed to submit complete and careful documentation to support the request. Such documentation was prepared

National Research Council	101,000.00	200,000.00
Canadian Dental Association	7,500.00	4,000.00
Fed. Nat. Health & Welfare	34,000.00	34,000.00
Other	7,000.00	7,000.00
Totals	\$149,500.00	\$225,000.00

and forwarded to the Executive of the National Research Council at the time the budget was being considered. Justification for the large increase in funds was based on the fact that dental research is continuing to grow in Canada; that more and more research workers are being added to the staff of the dental schools; that present grants were in general too low; that funds were needed to provide Associateships; that the serious shortage of money was inflicting unwarranted hardship on some workers. It was pointed out that this Committee felt it should continue to support research being conducted by workers other than dentists where the research was felt to be of interest and value to dentistry, and that if we were to continue to support present well-established programmes of dental research as well as to provide funds to enable new workers to become established, then more funds would have to be forthcoming.

"The National Research Council largely acceded to our request and the allocation for the Associate Committee on Dental Research for the present session was tentatively set at \$200,000. We are therefore in the unique position of having had our allocation almost doubled and of having received requests for less money than last year. Both the total number of applications and the total amount of money requested are down. Since there has been no voluntary reduction in the amount of dental research being conducted in Canada during the past year, nor presumably has there been any decrease in the desire to have research projects established, one can only surmise that the reason for the reduction in applications this session is because of the pessimism respecting available funds for research.

"In any case, programmes of expansion in several of the schools are just nicely getting started, and there is no reason to assume that the number of applications received this year is a true indication of what will be forthcoming. I have no doubt that in the relatively near future, this Committee will be faced with the problem of acute shortage of funds.

"Other Sources of Funds: Table I was prepared to familiarize Committee members with the place of the National Research Council in supporting dental research in Canada. This indicates in round figures the amounts available for dental research not only from the National Research Council, but from other sources as well, for both 1960-61 and 1961-62. Federal funds through the National Research Council are, and probably will remain, the predominant source of dental research funds in this country.

TABLE I
FUNDS FOR DENTAL RESEARCH

<u>Source</u>	<u>1960-61</u>	<u>1961-62</u>
National Research Council	100,000.00	200,000.00
Canadian Dental Association	7,500.00	9,000.00
Dept. Nat. Health & Welfare	34,000.00	34,000.00
Other	7,000.00	7,000.00
Totals	\$148,500.00	\$250,000.00

"Relation of Research Costs and Treatment Costs in Dentistry:
In order to see how dental research funds compare with those for medical research in Canada, and to compare both with the costs for treatment in dentistry and medicine, Table II has been prepared.

TABLE II
COST OF TREATMENT vs. COST OF RESEARCH
DENTISTRY AND MEDICINE
CANADA 1961 (POP. 20,000,000)

Cost of	Dental		Medical		Proportion Treatment/ Research	
	Total	Per Capita	Total	Per Capita	Dent.	Med.
Treatment	\$100,000,000.00	\$5.00	\$600,000,000.00	\$30.00	400/1	60/1
Research	250,000.00	0.0125	10,000,000.00	0.50		

Canadians now spend approximately \$5.00 per capita each year for dental treatment and 1-1/4¢ per capita to support dental research. These figures compare with \$30.00 per capita and 50¢ per capita for medical treatment and research respectively. This indicates that for every \$60.00 spent for medical treatment Canadians invest \$1.00 in medical research. In dentistry for every \$400 for treatment \$1.00 is invested in research. According to the recent survey of dentistry in the United States, in 1958 in that country approximately 5¢ per capita was available to support dental research. It is somewhat higher than that at the present time.

"This Table was not prepared as a criticism of the availability of money to support dental research in Canada. It was compiled to illustrate how much more building is necessary in research in dentistry in Canada if we are to compare favourably with research spending in the other health services in this country or elsewhere. At the moment I do not see how we could wisely use more money than we have this year.

(b) Expansion of Dental Research in Canada

"In any case, the simple provision of more money will not necessarily bring about an expansion - or more important - a healthy expansion, of dental research in Canada. In the last analysis this can only come about

through activity in the individual schools of dentistry. More men capable of doing research must be appointed to school staffs, and time must be provided for them to do research. There is evidence to indicate that these things are happening.

"At our last meeting it was suggested that on the basis of Fellowship awards which had been made by the Committee over the past several years, we might conceivably expect 4 additional research workers to be added to the Canadian pool each year, assuming of course that all those who have received N. R. C. Fellowships return to Canada.

"Within the last few weeks I have written to the Dean of each of the dental schools in Canada to ask the number of people presently engaged in research in each school, the number in training, and the number of research workers that might be considered a maximum for each school. On this basis it appears that about 30 research workers are active in Canadian dental schools to-day and this figure compares favourably to that disclosed by the survey of dental research in Canada conducted by the Canadian Dental Association two years ago. About 10 people are now taking research training, and within the relatively near future, assuming that the dental schools receive the support and assistance from the Universities that they will need, the number of research workers in dental schools may be doubled. This increase in numbers will probably happen within the next five to ten years.

"In addition we should expect an increase in the number of dental schools in Canada soon. During the last meeting of the Canadian Dental Association, a panel discussion was held on the subject of manpower in Canadian dentistry. As a member of this panel, Dean Ellis of the University of Toronto demonstrated that if Canadians were to continue with a dentist/patient ratio of 1/2,500, at least three new dental schools, each graduating 60-75 students per year, must be operating by the year 1978. The first of these schools would have to be completed and graduating students by 1968, the second by 1973 and the third by 1978. (J. C. D. A. January, 1961).

"A summary of this predicted increase in number of dental research workers has been presented in Table III, together with an estimate of the amount of money it will take to support them, based on estimates of \$7,500 and \$15,000 per worker per year. At present the Canadian figure is something less than the \$7,500 shown and that of \$15,000 per worker per year is that estimated by the Commission on the survey of dentistry in the United States as being necessary for research support.

"If these prognostications are even reasonably accurate, it can readily be seen that within the next 15 years Canadians should be investing well over \$1,000,000 per year in support of dental research.

TABLE III
FORECAST OF FUNDS NECESSARY
TO SUPPORT DENTAL RESEARCH
CANADA 1961-1978

Year	No. of Workers		Necessary Funds for Support	
	Expected	Total	\$7,500/worker	\$15,000/worker***
1961		30	225,000	450,000
Immediate * Increase	30	60		900,000
1968**	15	75		1,125,000
1973**	15	90		1,350,000
1978**	15	105		1,575,000

* Based on prediction of Deans of Dental Schools

** Ellis, R.G. J.C.D.A. 27(1):3, 1961

*** "Survey of Dentistry in the United States", 1960

(c) The Role of the Associate Committee on Dental Research in the Expanding Dental Research Programme in Canada

"If the experience over the past year may be taken as an example it seems obvious that when a real need for additional funds to support Canadian dental research can be demonstrated, it will be forthcoming. If accurate records of dental research activities are kept from year to year, and if plans for development in each of the schools are available, surely it will not be necessary to wait until the shortage of money is as acute as it was last year, before the need can be demonstrated.

"But besides finding funds for research support, this Committee can assist in the healthy expansion of research in the dental schools in other ways. As long as the National Research Council remains the chief source of research money this Committee will continue to screen more research applications than any other organization. Through the experience and knowledge of its own members, and through the use of external referees, we will have a most responsible role in maintaining high standards of quality in dental research in Canada. This role is perhaps all the more important this session when we are in the position of having an excess of funds.

"Schools could also probably use help in solving one of the chronic problems in research - finding time for research workers to do research. This problem was strongly emphasized in the survey of dentistry in the United States, which reported that nearly one quarter of the research workers in the dental schools surveyed spent less than 10% of their time in investigatory work. This situation is not easy to rectify when Deans are squeezed between University budget limitations and teaching requirements.

"However, this Committee could help to ease the problem in two ways: first, the wise appointment of Dental Research Associates would help increase the quantity of work being done in those schools to which such people were appointed, and may, at the same time, provide leadership in research activities which might otherwise be lacking. Second, as was briefly discussed at the Committee meeting last year, there is a need for a type of award that will provide interim support for new research workers while they are becoming established. In this relation I shall ask the Committee subsequently to consider the establishment of a new award that will provide adequate financial support for young research workers who have just completed their programme of graduate study."

Dr. Rhodes opened discussion on the Chairman's remarks. He expressed considerable surprise at the statement that there are thirty people engaged in dental research in Canada. It was noted that this figure is comparable to those disclosed by a survey made by the Canadian Dental Association in 1959. At that time there were 24 dental research workers, 3 of whom had the DDS degree only, the remaining 21 having the DDS degree and a basic science degree. The majority of them held senior staff appointments at universities.

In reply to a further question from Dr. Rhodes, the Chairman reported that in 1959 there were 6 holders of the D. D. S. degree undertaking graduate studies; all were proceeding to the Master's degree at the University of Toronto. In addition, there were approximately 15 graduates in Dentistry proceeding to certification in a clinical specialty; while research does not play a major role in these latter courses, some research training is given.

To Dr. Slater's question, the Chairman replied that the thirty workers now in dental research include not only those with the D. D. S. degree but also those whose training had been in the basic sciences. It does not, however, include scientists working in broader fields, such as that of collagen research, which may be of great interest and value in relation to dentistry but are not solely dental research per se.

5. Programmes at University of Alberta and Dalhousie University

The Chairman invited the Committee's guests to describe the programmes of their universities.

Dean MacLean of the Faculty of Dentistry at the University of Alberta reported that his university is at present in the process of increasing the science part of the school and it is expected that the physical facilities will be completed in June. The actual space will be increased from 13,000 sq. ft. to 56,000 sq. ft., not including the basic science departments, or library space. The plans include a fairly extensive area specifically for the accommodation of workers who expect to spend the major portion of their time in research; in addition, provision is made for research laboratories in conjunction with three offices and in the eight departmental offices there is a certain amount of space available where small laboratories can be constructed for the use of those who may wish to have research facilities near their offices. Excellent liaison is maintained with the members of the basic science departments. Animal quarters are provided on the roof which will be available for the use of research workers in the Faculties of Dentistry and of Medicine. Dean MacLean expressed the hope that, with the new facilities, the dental research programme at Alberta would soon show rapid development.

Dean J. McLean of the Faculty of Dentistry at Dalhousie University pointed out that Dalhousie is essentially a private institution and, in planning the new quarters for his Faculty, there had been some restriction on the amount of space which could be provided for research. Some space is available and being used, however, and as additional staff is appointed an effort will be made to make research space available to them. He noted that in the basic science departments of the Medical School at Dalhousie, which are also responsible for teaching dental students, there has been a genuine interest and some activity in dental research problems. There has already been a small increase in the amount of basic science space and considerable improvement in this regard is anticipated in the next four or five years.

6. Support for Dental Research Personnel

The Chairman outlined the methods currently available to the Committee for supporting dental research personnel:

Summer Assistantships - to enable dental students to work in research laboratories during the summer months under the direction of grantees of the Associate Committee on Dental Research;

Dental Research Fellowships - to enable holders of the D.D.S. degree to take training in research under the supervision of qualified investigators;

Graduate Assistantships - to enable young dental graduates to gain experience in research laboratories as employees under grants; and

Dental Research Associateships - to provide career opportunities for individuals who have shown the ability to conduct independent research.

He pointed out that there still seems to be no suitable means of supporting a dental graduate who has had some research training, possibly while holding a Dental Research Fellowship, but who has not yet had the opportunity to show an ability to conduct independent research. He is therefore not qualified for an Associateship and it is difficult to find appointments for such individuals in dental schools.

The Chairman suggested that an intermediate category of "Junior Associate" be established for such individuals. He circulated a draft proposal for such a category. In general, it would be similar to the normal Dental Research Associateship, i. e. , it would be a full-time research appointment, supernumerary to the university's staff complement, with provision made for the incumbent's salary, his employee benefits and a grant of \$2,000 towards his research during the first year. The proposed category, however, differed from current Associateship regulations in that, after an initial appointment of two years, the case would be carefully reviewed, and if the university wished to do so it could recommend renewal of the appointment on a part-time basis which would commit the university to assuming increasing responsibility for the incumbent's salary over the next three years; at the end of that time he would be a full member of the university staff, devoting part-time to teaching and part to research.

The Chairman felt that the establishment of such a category of research support would do much to enable a young investigator to get his research going without being hampered by teaching duties, and would also give the universities an opportunity of making a better assessment of the person's qualifications. Moreover, the gradual assumption of responsibility for the person's salary would make it easier for the dean of a dental school to obtain funds for his eventual complete support.

Dr. Scott noted that when the new proposal had been discussed with Dr. Marshall, the latter had suggested the possibility of extending the current regulations governing Dental Research Associateships to include provision for such a category. Dr. Scott agreed with Dr. Marshall that this might be less confusing than the establishment of a completely new category of appointment.

After some further discussion, it was MOVED by Dr. Kleinberg and SECONDED by Dr. Francis,

THAT the principle as outlined by the draft proposal of the Chairman be adopted.

CARRIED

At the suggestion of Dr. Slater, it was AGREED that the Chairman and Dr. Gornall should consider the possibility of integrating the draft proposal for "Junior Associates" with the current regulations governing Dental Research Associateships, and report back to the Committee (See Minute 15, below).

7. Reports from Grantees

The Chairman informed the Committee that, due to the heavy demands made on the duplication services of the National Research Council, it had not been possible this year to send copies of grantees' reports to all members prior to the meeting as was done in previous years. The reports had in most cases, however, been reviewed by the referees to whom renewal applications had been sent.

He asked the Committee if it would be desirable to continue having grantees' reports reproduced as Appendices to the Proceedings of the meetings. It was generally agreed that since there is as yet relatively little dental research being done in Canada, it is useful for all members of the Committee to have full information on the work supported; reports should therefore continue to be included in the Proceedings. Reports on projects for which applications for renewal had been submitted are therefore appended as follows:

<u>Grantee</u>	<u>Project No.</u>	<u>Appendix</u>
Dowse, C. M.	DA 81	B
Hunt, A. M.	DA 82	C
Kleinberg, I.	DA 78	D
Leblond, C. P.	DA 23	E
Mezl, Z.	DA 79	F
Trott, J. R.	DA 85	G
Copp, D. H.	DT 59	L

Dr. Gornall suggested that grantees be asked to submit not only a report on their year's work but a one-page summary of the report. Copies of the summaries could then be sent to all members of the Committee

prior to the meeting. It was AGREED that this would be done in future. Reviewers would, of course, continue to receive the full report on any project for which a renewal application had been submitted.

Dr. Belanger asked if any thought had recently been given to resuming the previous practice of inviting two or three grantees to attend the meeting of the Committee to present reports on their work; he had found these sessions most stimulating and, since there are so few opportunities to discuss dental research problems with other workers in Canada, they served a very useful purpose.

The Chairman said that this practice had been discontinued because administrative and policy matters had demanded more attention in the last two or three years; he suggested, however, that if meetings were to be held twice a year, such scientific sessions might again be practicable. Dr. Gornall, however, expressed the view that the funds of the Committee might better be devoted to direct support of research, rather than to meetings of the grantees.

The Chairman reported that reports had been received from five grantees who had not re-applied for further support in 1961-62:

- (a) Project DT-52, Dr. L. F. Belanger: "The intimate mechanism of mineralization". (See Appendix K)
- (b) Project DA-69, Dr. K. A. McMurchy: "The study of traumatic tooth movements". (See Appendix H)
- (c) Project DA-75, Dr. K. A. McMurchy: "Histopathology". (See Appendix M)
- (d) Project DA-83, Dr. F. J. Marshall: "In vivo studies of dentine permeability and root canal filling seals with radiosulfur" (See Appendix I)
- (e) Project DA-74, Dr. A. E. Hoffman: "The use of an anorganic bone graft following partial mandibular re-section in the rat". (See Appendix J)

Dr. Neilson reported that Dr. Marshall had had difficulties getting his research organized and since a portion of his 1960-61 grant was still available to him he had not found it necessary to re-apply at this time.

Dr. McLean said that Dr. Hoffman also had sufficient funds to continue his project during the coming year.

Dr. Belanger reported that he had not applied for a renewal of his term grant because his research is not at the moment directly related to dental problems; moreover, he had anticipated that the funds which would be available to the Committee would be sufficient to support only projects of immediate dental interest.

8. Meeting of the Executive

The Chairman informed the Committee that the members of the Executive (Dr. G.D. Scott, Dr. A. D'Iorio, Dr. A.G. Gornall, Dr. I. Kleinberg, in addition to the Chairman) had met on the afternoon of February 22nd. Dean H. MacLean and Dean J. McLean had also attended the meeting as guests. Preliminary consideration had been given to the applications for Fellowships and for grants in aid; the recommendations of the Executive would be submitted to the Committee as each application was reviewed.

9. Financial Position of the Committee

The Chairman informed the Committee that its allotment for the year 1960-61 had been almost entirely spent, leaving only sufficient for payment of the cost of the current meeting.

In connection with the allocation for 1961-62, which had initially been set tentatively at \$200,000, the Chairman noted that the total amount of applications submitted for 1961-62, in all categories, was less than \$140,000.

In view of the great demands made on Council in other disciplines, it would obviously be unreasonable for the Dental Committee to expect to be able to hold all of \$60,000 for which applications had not been received. The original allotment had been reduced to \$140,000, although further funds might be forthcoming if they were definitely needed.

Dr. Francis suggested that grants made last year in amounts less than those applied for might now be raised since funds were available. The Chairman said, however, that consideration could be given to increasing grants only if applications for supplementary amounts were received; any such applications which might be received would be considered in relation to each other and in the light of the funds still available to the Committee.

10. Applications for Fellowships

Four applications for Dental Research Fellowships tenable during 1961-62 were considered: three for the renewal of current Fellowships and one for a first award. Action was taken as follows:

Blackmore, R. V.	To work in the Dept. of Bacteriology, Strong Memorial Hospital, University of Rochester, under Dr. H. R. Morgan (renewal)	APPROVED \$5,000
Johnston, M. C.	To work in the Dept. of Dentistry and Dental Research, University of Rochester School of Medicine & Dentistry, under Dr. E. Johansen (renewal)	APPROVED \$5,000
Listgarten, M. A.	To work in the Harvard School of Dental Medicine, under Dr. P. Goldhaber (renewal)	APPROVED \$3,750
Taichman, N. S.	To work in the Harvard School of Dental Medicine, under Dr. P. Goldhaber	APPROVED \$3,000

Dr. Rhodes noted that all the Fellowships are to be held in laboratories outside Canada. The Chairman explained that there are very few programmes in Canada leading to graduate degrees in dentistry, although he hoped that more such facilities would soon become available in this country.

Dr. Rhodes suggested that the Committee might do well to consider devoting a portion of its appropriation to attracting to Canada senior investigators highly qualified in dental research who could contribute to the development of postgraduate programmes in this country. The Chairman said that most dental schools do not have a staff establishment that would permit the employment of research workers qualified in the basic sciences; they have generally been able to hire only staff for dental teaching and have had to depend on the basic science departments in other faculties for instruction of dental students in those fields. With the current upsurge in research activity, however, it is gradually being recognized that it is advisable to have teachers in the basic sciences as members of the normal staff of a dental school. The problem is two-fold: there is a need to interest dental graduates in the basic sciences and also a need to interest basic scientists in research related to dentistry.

Several members felt that the proposed expansion of the Dental Research Associate programme would be of considerable assistance to the Dental Schools in providing opportunities for the initiation of research programmes by young investigators who could subsequently be absorbed into the normal staff establishment.

11. Applications for Grants, 1961-62

The Chairman noted that funds were already committed for the coming year for term grants as follows:

Burgen, A.S.V. Physiology, McGill	Studies on protein secretion and other processes in the salivary glands DT-61	\$6,000 the third instalment of a three-year Term Grant
Francis, L.E., Pharmacology, McGill	Studies on gingival changes due to drug administration DT-76	\$5,000 the second instalment of a three-year Term Grant
Lussier, J.-P., Dental Surgery, Montreal	Studies on citrate metabolism in bone and teeth as affected by hormonal and nutritional factors DT-70	\$4,800 the third instalment of a three-year Term Grant
Paynter, K.J., Dental Research, Toronto	The relation of nutrition to morphology and caries sus- ceptibility in the teeth of rats DT-72	\$5,000 the third instalment of a three-year Term Grant

Dr. G. Nikiforuk had been granted \$5,000 as a three-year Term Grant, beginning 1 April 1960, in support of his project "Organic compounds of dental tissue". He had, however, not yet been able to undertake this project and had therefore requested permission to carry forward to 1961-62 the funds awarded last year. Permission had been granted and the second instalment of the Term Grant was deferred for payment in 1962-63.

Twenty-one grant applications were then considered. Most of them had been reviewed before the meeting by scientists qualified in the particular field involved, and copies of the reviewers' comments were available to all members of the Committee.

<u>Applicant</u>	<u>Title and Amount Requested</u>	<u>Recommendation</u>
Anderson, D.L., Dental Research, Toronto	Histochemical investigation of cell cytoplasm and inter- cellular interfibrillar material DA-86 \$2,000.00	Approved \$2,000

<u>Applicant</u>	<u>Title and Amount Requested</u>	<u>Recommendation</u>
Copp, D.H., Physiology, Br. Columbia	Phosphorus deficiency: Effects on bones and teeth DT-59 \$6,400.00	Approved \$6,400 as three-year Term Grant
Dowse, C.M., Oral Biology, Manitoba	Metabolism of periosteum DA-81 \$7,816.00	Approved \$4,840 Not Approved
Findlay, J., Periodontics, Dalhousie	An investigation into the effects of an artificially in- duced oestrus cycle on the periodontal tissues of hypo- physectomised rats DA-87 \$1,824.00	Approved \$524 (Student assistant requested to be con- sidered for Summer Assistantship)
Francis, L.E., Clinical Dentistry, McGill	The storage medium for a functional tooth bank: A study on methods of storage and procedures for evaluation DA-88 \$3,000.00	Approved \$1,600 of which \$1,000 was provided for the salary of R. J. Coburn
Grainger, R.M., Dental Research, Toronto	Statistical consultation and analysis unit DT-89 \$6,538.60	Approved \$2,500 Approved \$7,000 as a three-year Term Grant
Hunt, A.M., Dental Research, Toronto	Measurement of strontium ⁹⁰ and carbon ¹⁴ in primary teeth of Canadian children DA-82 \$5,800.00	Approved \$5,800
Kasloff, Z., Dentistry, Manitoba	The relationship of defects in teeth associated with in- duced stresses to the histo- logical pattern of tooth structure DA-90 \$5,950.00	Approved \$5,250 including an amount of \$350 to enable grantee to visit lab. of A. F. Forziati in Washington
Kleinberg, I., Biochemistry, Manitoba	1. Further studies on the role of the dental plaque in the caries process. 2. Further studies on the effect of substances removed during the refining of carbo- hydrates on calcium phosphate solubility DT-78 \$6,598.00	Approved \$6,600 as three-year Term Grant

<u>Applicant</u>	<u>Title and Amount Requested</u>	<u>Recommendation</u>
Kropp, B.N., Histology & Embryol. Queen's	The influence of fluoride on differentiation of calcified structures and on terato- genesis DA-91 \$4,385.00	Approved \$4,385 (Student assistant requested to be considered for Summer Assistantship)
Kropp, B.N., Histology & Embryol. Queen's	The effects of growth accel- erators on odontogenesis DA-91 \$3,931.00	Not Approved
Leblond, C.P., Anatomy, McGill	Entry of carbohydrates and proteins into teeth as shown with the help of radioactive elements DA-23 \$4,400.00	Approved \$4,400
Lussier, J.P., Dental Surgery, Montreal	Chromatogram scanner and accessories DE-12 \$1,800.00	Approved \$1,800
Mezl, Z., Dental Surgery, Montreal	Penetration of minerals from saliva and shifting of minerals in the carious lesion DA-79 \$2,500.00	Approved \$2,500
Paynter, K. J., Oral Anatomy, Toronto	The relation of nutrition to morphology and caries sus- ceptibility in the teeth of rats DT-72 \$3,500.00 (Supplementary)	Withdrawn
Poyton, H.G. and Torneck, C.D., Radiology, Toronto	Photovolt electronic densi- tometer DA-96 \$405.00	Approved \$475 (the cost of the equipment was known to have recently increased)
Sinclair-Hall, A.H., Anatomy, Manitoba	Polyvinyl-alcohol sponge and the healing of bony defects DA-92 \$1,200.00	Approved \$1,200

<u>Applicant</u>	<u>Title and Amount Requested</u>	<u>Recommendation</u>
Spouge, J. D., Oral Biology, Manitoba	An investigation into the relative roles of intermittent lateral stresses applied to the teeth, and administration of a soft but nutritionally adequate diet, in the production of periodontal disease in the jaws of dogs DA-93 \$1,920.00	Approved \$1,120 (Student assistant requested to be considered for Summer Assistantship)
Trott, J. R., Oral Biology, Manitoba	An histochemical investigation of the glycogen content in the developing gingiva DA-85 \$3,550.00	Approved \$3,550
Tuba, J., Biochemistry, Alberta	The role of metaphosphoric acid in dental caries in the hamster DA-94 \$7,156.00	Approved \$6,250
Youdelis, W. V., Mining & Metallurgy, Alberta	Fundamentals of amalgam metallurgy and the development of dental amalgams DA-95 \$5,240.00	Approved \$5,240

It was agreed that the reviewers' comments on the applications of Dr. Anderson, Dr. Mezl, Dr. Trott, Dr. Tuba and the two applications of Dr. Kropp should be sent to the applicants for their information.

The number of referees to be asked to comment on each application was discussed. Dr. Gornall felt that since the number of applications is still small, the Committee is able to scrutinize each one rather closely. It therefore seemed to him that one referee was adequate in each case. Dr. D'Iorio suggested that if a reviewer recommended that an application be rejected, the opinion of a second reviewer might be sought. It was agreed that this could be done in future.

Dr. Gornall also suggested, and it was agreed, that the Personal Data forms of the applicants be sent to the reviewers with all applications.

There was some discussion as to the procedure for dealing with additional applications which may be submitted. It was AGREED that, if

only a few additional applications are received, the Executive might meet to consider them. If the number of additional applications appears to warrant it, another meeting of the full Committee might be called, or the Executive's recommendations might be circulated to all members of the Committee for review before additional grants are made. The decision as to the procedure to be followed was left to the discretion of the Chairman.

12. Application for Travel Grant

Dr. F. J. Marshall of the Faculty of Dentistry, University of Manitoba, had submitted an application for \$650 to enable him to take a six weeks' course on "Radioisotopes in Research" at the Oak Ridge Institute of Nuclear Studies, Oak Ridge, Tennessee.

The Committee did not feel that it could approve such a request, but suggested that Dr. Marshall be advised to apply for funds to the Research Committee of the Canadian Dental Association.

13. Summer Dental Research Assistants

Posters had been sent to all Dental Schools in the Fall, advertising the summer assistantships made available by the Committee to enable dental undergraduates of high academic calibre to work in research laboratories during the summer. For administrative simplicity, these awards are tenable only in the laboratories of the Committee's grantees.

Applications this year had been submitted directly to a Committee representative in each of the various dental schools; after preliminary screening in the schools, the names of those who were judged most suitable, and could be accommodated by a grantee, were submitted to the Committee for consideration.

For the benefit of new members, the Chairman explained that the salaries paid to successful applicants for assistantships depend on the year of academic training: \$265 per month for those who have just completed the first year of the dental course, \$285 for those completing their second year and \$305 for those completing their third year. The Chairman noted that this scale of payment is the same as that paid to science and engineering undergraduates employed during the summer months in the N. R. C.'s laboratories.

Several members of the Committee took exception to this rate, since it is considerably higher than that permitted for graduate students. It was pointed out that the rate offered to undergraduates must compare favourably with the wages obtainable in construction or labouring jobs.

Graduate students, on the other hand, are engaged during the summer in furthering their own research; in this case the element of competition is related to the number of students seeking support rather than related to the labour market.

After some further discussion, it was AGREED that the current rates for Summer Dental Undergraduate Research Assistants be maintained for this year as advertised, but that the Executive should discuss with the National Research Council the possibility of reviewing the scale of payment for summer employment.

The duration of Summer Dental Research Assistantships was also discussed. In previous years, the Committee's budget had made it impossible to employ such assistants for more than three months; other sources of funds had to be relied upon if the services of an Assistant were to be retained for a longer period. After some consideration, it was AGREED that for the current year, the Committee might provide an Assistant's salary for up to four months, if this amount of time were available to the student concerned. This point, too, should be reviewed before awards are advertised next year.

After review of the individual cases, it was AGREED that the following Assistants be appointed for the coming summer; and the amounts of their salaries be added to the 1961-62 grants of the designated supervisors:

Alberta

Grigoruk, A.	To work with Dr. J. Tuba and Dr. K. McMurchy	3 mos. @ \$305 \$915
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Dalhousie

Paul, I.	To work in the laboratory of Dr. J. Findlay	3-1/2 mos. @ \$285 \$997.50
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Logue, J.	To work in the laboratory of Dr. A.E. Hoffman	4 mos. @ \$305 \$1,220
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Manitoba

Trester, P.H.	To work in the laboratory of Dr. I. Kleinberg	3-1/2 mos. @ \$305 \$1,067.50
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Kaufman, H.W.	To work in the laboratory of Dr. I. Kleinberg	3-1/2 mos @ \$285 \$997.50
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Chesko, E.	To work in the laboratory of Dr. C. W. Dowse	3-1/2 mos. @ \$265 \$927.50
Gorenstein, S.	To work in the laboratory of Dr. J. R. Trott	3-1/2 mos @ \$285 \$997.50
Diamond, H.	To work in the laboratory of Dr. J. D. Spouge	3-1/2 mos. @ \$285 \$997.50

Montreal

Lebuis, J. J.	To work in the laboratory of Dr. J. P. Lussier	3-1/2 mos. \$265 \$927.50
Lemieux, R.	To work in the laboratory of Dr. Z. Mezl	3-1/2 mos. @ \$305 \$1,067.50
Bing, H. R. E.	To work in the laboratory of Dr. G. Nikiforuk	3-1/2 mos. @ \$285 \$997.50
Cross, R. G.	To work in the laboratory of Dr. A. M. Hunt	4 mos. @ \$265 \$1,060
Direnfeld, V. N.	To work in the laboratory of Dr. K. J. Paynter	3-1/2 mos. @ \$285 \$997.50
Girdlestone, J. G.	To work in the laboratory of Dr. G. Poyton	2 mos. @ \$305 \$610

McGill

Abood, J. G.	To work in the laboratory of Dr. A. S. V. Burgen	3 mos. @ \$305 \$915
Gibbons,	To work in the laboratory of Dr. C. P. Leblond	3 mos. @ \$285 \$855
Smallwood, W. C.	To work in the laboratory of Dr. C. P. Leblond	3 mos. @ \$305 \$915
Yeung, E.	To work in the laboratory of Dr. L. E. Francis	3 mos. @ \$305 \$915

14. Summary of Recommended Expenditures

It was therefore AGREED that the following expenditures be recommended to Council at this time:

4 Fellowships	\$16,750
7 Term Grants	40,800
14 Annual Grants	48,659
2 Equipment Grants	2,275
18 Summer Assistantships	17,380

In addition, the amount of \$2,000 should be set aside for Committee expenses for 1961-62.

15. Dental Research Associates

The Chairman reported that, as instructed by the Committee (see Min. 6 above), he and Dr. Gornall, in collaboration with Dr. J. B. Marshall, had attempted to revise the regulations governing Dental Research Associateships to incorporate the recommendations of the Committee. A draft of the revised regulations was distributed and read (See Appendix A).

Dr. Belanger questioned the advisability of limiting to 40 the age of candidates for initial appointment as Associates. He felt that such appointments might profitably be made available to highly qualified investigators who had reached the university retirement age but could still make very useful contributions to the development of new research programmes. It was generally felt, however, that the appointment of superannuated scientists would not be permissible, in view of Government regulations concerning retirement at age 65.

Dean Neilson questioned the stipulation that candidates for Associateships should be Canadian citizens or residents; he felt that much could be gained if such appointments were open to highly qualified research workers in other countries. The Chairman pointed out that the emphasis should be on the appointment of young Canadians, for whom it is now difficult to get university posts; university budgets are often established several years in advance and therefore do not permit the employment of younger men at the time when their research training has just been completed. Associateships would, however, also be open to qualified research investigators domiciled in Canada with the intention of remaining here.

With regard to the initial salary of an Associate, it was pointed out that it would depend on the qualifications and experience of each appointee. No Associate, however, would receive less than \$7,050 per annum (the present starting salary for a new Ph. D. at the National Research Council).

Dean McLean asked if a new Associate would be free to apply to the Committee in his first year of appointment for an equipment grant, or whether he would be limited to the \$3,000 outright grant which is automatically given to each Associate to enable him to get his work started. It was AGREED that the initial grant from the National Research Council should be limited to \$3,000 for the time being, but the matter might later be reviewed in the light of experience.

It was noted that the regulations relating to tenure had been revised to make possible the gradual assimilation of an Associate into the normal staff complement of the university if this became desirable. Each such case would be considered individually and appropriate measures taken to transfer full responsibility for the Associate's salary from the National Research Council to the university concerned; it was agreed, however, that it would in general be desirable if the transfer could be completed within a three-year period following the decision to make such a transfer.

It was MOVED by Dr. Nikiforuk and SECONDED by Dr. Kleinberg THAT the revised regulations for Dental Research Associates be accepted and forwarded to the National Research Council for approval.

CARRIED

16. General Research Grants to Deans

The Chairman noted that the new Medical Research Council plans to give some consideration to the provision of general research grants to Deans of Medical Schools on the same basis as the grants currently being made to University Presidents by the National Research Council. He did not feel, however, that the Committee would take any action with regard to recommending research grants to Deans of Dental Schools; at the present time, the Dental Schools can still benefit from the general research grants to University Presidents. The Medical Research Council on the other hand is now an autonomous body and presumably Deans of Medical Schools cannot therefore expect to benefit to the same extent from National Research Council grants to University Presidents.

17. Membership of the Committee

The Chairman informed the Committee that the term of appointment of Dr. A. D'Iorio and of Dr. R. L. deC.H. Saunders would expire on 31 March 1961. According to the Council's regulations governing Committee

membership, no member may serve more than two consecutive three-year terms. Dr. D'Iorio and Dr. Saunders had each completed one three-year term on the Committee.

Dr. D'Iorio pointed out that while he had been at the University of Montreal he had been able to represent that University on the Committee; since he had now joined the staff of the University of Ottawa, he did not feel that his present term should be extended.

It was MOVED by Dr. D'Iorio and SECONDED by Dr. Francis

THAT Dr. J. -P. Lussier of the University of Montreal be recommended to Council for appointment to the Committee.

CARRIED

It was MOVED by Dr. Castaldi and SECONDED by Dr. Belanger

THAT Dr. R. L. deC.H. Saunders of Dalhousie University be recommended for re-appointment to the Committee for a further three-year term.

CARRIED

The Chairman noted that it would be necessary to have a replacement for Dr. D'Iorio on the Committee's Executive. It was AGREED that the Executive for 1961-62 should consist of Dr. Paynter (Chairman), Dr. A.G. Gornall, Dr. I. Kleinberg, Dr. J. -P. Lussier and Dr. G.D. Scott.

On behalf of the Committee, the Chairman expressed regret that Dr. D'Iorio was retiring from the Committee, and thanked him for his valuable service as a member of both the Committee and the Executive

There was brief discussion as to the value of having, as an ex officio member of the Committee, the Chairman of the Canadian Dental Association's Council on Dental Education. It was pointed out that the Chairmanship of the Council on Dental Education is also a three-year appointment and the incumbent could therefore be expected to fulfil a useful liaison capacity between the Associate Committee on Dental Research and the Canadian Dental Association.

It was MOVED by Dr. Paynter and SECONDED by Dr. Nikiforuk

THAT it be recommended to Council that the Chairman of the Canadian Dental Association's Council on Dental Education be appointed an ex officio member of the Associate Committee on Dental Research.

CARRIED

18. Estimates for 1962-63

There was some discussion of the amount which could reasonably be requested by the Committee for 1962-63. It was noted that the number of grant applications for 1961-62 had fallen far short of expectations. This could be explained in part by the fact that some grantees had not been able to get their projects started as early as had been anticipated; since unexpended funds could be carried forward for use in 1961-62, they had not found it necessary to re-apply this year.

The main reason for the lack of applications, however, was felt to be directly attributable to the drastic action which the Committee had found necessary last year; the reduction to the bare minimum of grants that were approved, and the rejection of others that were worthwhile but could not be supported because of lack of funds. As a result, applicants had been unduly pessimistic about the probability of getting support.

It was generally agreed that research investigators should submit applications for the support they really feel is needed, and be prepared to accept the amount that can be made available to them. Only in this way can the desired steady growth of dental research in this country be achieved.

It was pointed out that the revision of the Dental Research Associateship regulations, if approved by Council, would likely result in a considerably greater demand for funds for personnel support.

After some further discussion, it was AGREED that the Committee should submit a request for the same amount for 1962-63 as it had requested for 1961-62, i. e., \$225,000.

19. Date of the Next Meeting

Unless the Chairman felt it necessary to call a meeting to consider further applications, the next meeting of the Committee would be held at approximately the same time next year.

The meeting adjourned at 1:15 p. m., on February 24th.

NATIONAL RESEARCH COUNCIL OF CANADA

DENTAL RESEARCH ASSOCIATES

In an effort to encourage the long term planning and development of dental research in Canadian universities, the National Research Council will provide funds for the salaries of a limited number of Dental Research Associates. Appointments in this category may be made to individuals with suitable ability and training who wish to make research a full-time career.

Applications should be made not by the prospective candidate but by the President or Principal of the university (on the recommendation of the Head of the Department concerned and the Dean of the Dental Faculty), who must undertake to provide adequate research facilities and to give the Associate an appropriate honorary or other academic rank on the staff of the Dental School or Faculty, in keeping with the practice of the university. An Associate is not to be regarded as an extramural research worker of the National Research Council.

Tenure: The first appointment of a Dental Research Associate will be for a period of two years. It may be renewed for five-year periods, but not beyond the normal retiring age of the National Research Council or the university concerned.

If at any time the University wishes to appoint the Associate to its regular academic staff, the transfer of responsibility for his salary may be negotiated with the National Research Council.

The appointment may be terminated at any time, for good and sufficient reason, by the appointee, the university or the National Research Council.

Qualifications: Two types of candidate may be eligible for an Associateship: (a) a candidate with a D.D.S. degree or its equivalent who, in a period of post-graduate training, has shown promise of ability to conduct independent research; (b) a candidate with a doctorate in one of the sciences related to dentistry.

Under ordinary circumstances, candidates should be under 40 years of age.

Applications may be submitted on behalf of Canadians, whether in Canada or abroad, or on behalf of candidates of other nationalities domiciled in Canada with the intention of remaining here.

Salary: The initial salaries of Dental Research Associates will be similar to those paid to National Research Council Research Officers of comparable competence, training and experience. (The present starting salary for a new Ph. D. scientist in the National Research Council is \$7,050, allowance being made for additional experience and proven research ability.) Salary payments will be made through the business office of the university and will be subject to income tax. Funds will be provided for the stipend and the contributions made on behalf of the Associate to the university's pension scheme and other benefits in which university staff members normally participate.

Emolument from other sources: A Dental Research Associate may engage in teaching or consulting practice to a limited extent. Routine teaching should be limited, but there need be no restriction on the amount of time spent in the supervision of graduate students since this activity is considered part of his research programme. Consultations by an Associate in clinical fields must be limited to work related to his research problem. Remuneration may be accepted for these services by an Associate, but the total amount must not exceed \$2,000 per annum.

Research grant: In the first year of his tenure, the Associate will receive from the National Research Council a grant of \$3,000 in support of his research; in subsequent years he may apply to the Council or other granting bodies for funds required to prosecute his research.

Appointment: Recommendations for appointments will be made by a Selection Committee named by the National Research Council. If applications are made by a university on behalf of more than one candidate in a single year, the candidates should be listed in order of preference. The Selection Committee need not be ruled by the university's list, but will be guided by it.

Applications: Applications must be made on the approved forms, and forwarded by the President or Principal of the university to reach the Awards Office, National Research Council, Ottawa 2, Ontario, not later than 15 January.

APPENDIX B

Progress Report to the Associate Committee on Dental Research

National Research Council

December 1960

Subject: Metabolism of periosteum

Grantee: C.M. Dowse

Project: DA - 81

A report, covering in detail the results obtained in previous work and during the first month of this project was given to the Gordon Conference on Bones and Teeth in July of this year. The principal points noted were the following.

The Q_{O_2} of calvarium preparations, 18.4 μ moles/gm. wet wt., was quite low relative to most tissues and was similar quantitatively to values reported in the literature for metaphysical preparations. It was unaffected by the addition of any of a wide variety of substrates except succinate. It was stable for many hours with no substrate added. The endogenous substrate utilised was shown to be glycogen and it proved impossible to deplete the cellular stores of this by prolonged pre-incubation with aeration.

The use of malonate as an inhibitor gave no evidence of the presence of a functioning TCA cycle as the slight reduction in the Q_{O_2} produced upon addition could not be reversed by TCA intermediates after succinate. Fluoroacetate addition reduced the Q_{O_2} some 25% at the 10 or 20 mM level and led to the accumulation of citric acid in the tissue, suggestive evidence of the presence of the TCA cycle, as was the increase in citrate accumulation produced by the presence of fumerate and pyruvate.

The tissue exhibits a marked aerobic glycolysis, and, in the presence of glucose, the uptake of the latter substrate can be accounted for completely by the lactic acid produced, indicating the continued utilization of an endogenous supply of substrate to maintain the Q_{O_2} .

The addition of parathyroid hormone in vitro produced a 20-25% increase in net lactic acid production whether glucose was present or not but only under aerobic conditions.

Further work during the year has been concerned with corroborating and expanding these earlier findings using, through force of circumstances, several different strains of rat. The results have been consistent. Also methods needed to study in more detail the metabolism of carbohydrate in the tissue are being developed. Progress has been made in utilizing gradient elution with formic acid from Domex columns for the separation of TCA intermediates in the tissue. The effect of varying concentrations of iodoacetate on glycolysis has been investigated more fully with the intention of using this inhibitor in conjunction with variously labeled glucose - C^{14} in looking for the hexose monophosphate pathway in the tissue.

APPENDIX C

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH NATIONAL RESEARCH COUNCIL

December, 1960

Subject: Measurement of Strontium⁹⁰ and Carbon¹⁴ in Primary
Teeth of Canadian Children.

Grantee: A. M. Hunt

Project: DA-82 Amount Awarded: \$4500.00 1960-61

Introduction

This project was undertaken in an attempt to provide a more accurate estimate of the discrimination against strontium⁹⁰ as it passes from cows to milk to humans by reducing the large errors due to metabolic exchange which are present when bone samples are used. Since the elements incorporated in tooth tissue are relatively stable, and because the time interval during which these elements are invested in primary teeth can be estimated accurately, dental tissues should give a more reliable indication of the uptake of strontium⁹⁰. The experiment should give information on possible differences in uptake of strontium⁹⁰ by bone, dentin and enamel. In addition, information will be obtained from widely scattered areas in Canada where wide variations in radioactivity have been found in food stuffs.

SUMMARY OF WORK

In 1960, experimental work was confined to the collection of teeth from different provinces and to the standardization of chemical procedures for the preparation of pure dentin and enamel samples, for the removal of strontium⁹⁰ and yttrium⁹⁰ and for determining the amounts of calcium and non-radioactive

strontium. The measurement of carbon¹⁴ will not be attempted at present until the number of teeth collected from each area is sufficient to yield enough carbon for analysis.

Tooth Collection

Samples of sufficient size for strontium⁹⁰ analysis have been received from Vancouver and Nova Scotia for the years 1952 to 1954. Teeth and bone from Ontario calves have been obtained for the years 1958 to 1960, as well as cattle teeth from 1955. The Montreal Baby Tooth Survey which is beginning to collect teeth from Montreal for the St. Louis study¹ will direct part of its sample here for analysis. Since the collection is slow in other provinces, some thought has been given to the establishment of a collection agency sponsored by a national women's organization. In addition, an appeal has been sent out to Canadian dentists through the Canadian Dental Association Journal.

Preparation of Samples

When an adequate sample of teeth was received from an area for a certain year, they were divided into two groups: central and lateral incisors, and cuspids and molars. These were processed separately because of the difference in their developmental chronology.

Analytical Procedures

Pure dentin and enamel were obtained by the acetone-bromoform flotation method of Manley and Hodge², and these were ashed at 900°C. Ten ash samples have been prepared.

Three analytical methods have been used for separation of strontium⁹⁰ as reported by Martell³, Grummitt and Milton⁴,

and Bryant, Morgan and Spicer⁵. The latter method is being perfected to give reproducible yields of strontium⁹⁰ and its daughter yttrium⁹⁰.

A spectrophotometric method for calcium and the method of Jury, Webb and Webb⁶, for non-radioactive strontium analysis using the flame photometer are being standardized.

Counting Procedures

Application has been made to the United Nations Committee on the Effects of Atomic Radiation and to the Health and Safety Laboratory of the U.S. Atomic Energy Commission for calibrated strontium⁹⁰ standards to calculate the efficiency of the gas flow geiger counter now in use. This counter is acceptable for the very active calf dentin samples but a low background counter with an anti-coincidence guard tube must be purchased in order to detect the low activities present in human teeth. Dr. P.G. Mar of the Radiation Protection Division of the Department of National Health and Welfare, Ottawa, has determined the strontium⁹⁰ activity in our sample of 1955 cattle dentin and reported that $14.28 \mu \mu c$ of strontium⁹⁰/per gram of calcium were present. Using the same 1955 dentin, chemical separations in this laboratory yielded a sample with high activity but no comparison can be made until suitable standards are available.

Discussion

Three other groups of investigators are engaged in similar projects in Great Britain and the United States. Bryant, Henderson, and Holgate⁷ have determined the uptake of strontium⁹⁰ in 13 samples of permanent bicuspid and

third molars from children 9 to 15 years of age and young adults 17 to 23 years of age. The strontium⁹⁰ content ranged from 0.1 to 0.4 $\mu\mu\text{c}$ strontium⁹⁰ per gram of calcium. Butler⁸ has reported on 5 samples of primary teeth and 13 samples of permanent teeth of humans from 4 to 31 years of age. Activities ranged from 0.04 to 2.8 $\mu\mu\text{c}$ strontium⁹⁰ per gram of calcium. Wyant¹ has collected large samples of primary teeth from St. Louis, Mo., and Montreal, P.Q. The latter study should be comparable in many ways to the one in Toronto.

The peak atmospheric content of strontium⁹⁰ was reached in 1960. Primary incisor and molar teeth developing during this period will be exfoliated between 1966 and 1972. It would be desirable to continue these studies until the above dates when a downward trend in strontium⁹⁰ content of teeth should occur.

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Report to the Associate Committee on Dental Research, National Research Council
on Project D.A. 78

Grantee: I. Kleinberg

Project I

Further Studies on the Role of the Dental Plaque in the Caries Process.

1. The paper entitled "Studies on dental plaque. The effect of different concentrations of glucose on plaque pH in vivo." is now in press (J. dent. Res.).
2. The study reported previously (see 1960 report), on whether the difference in bacterial cell concentration in plaque in vivo and saliva in vitro could account for the difference in the shape of the glucose concentration-pH curves for these two systems, has been extended and has now been completed. At the time of the previous report, it was thought from the observations then available that higher glucose concentrations had a greater inhibitory effect on bacterial growth than on bacterial acid production. However, additional experiments have clarified this point and do not support this earlier conclusion.
3. Since these glucose concentration-pH curves for saliva-glucose mixtures in vitro and for plaque in vivo could be made similar by increasing the cell concentration of the former, it was decided to carry out experiments to see if the various types of pH curves which can be observed in vivo with glucose (see 1959 report) could also be reproduced in vitro. By utilizing saliva mixtures containing high cell concentrations (lower levels were also studied) and altering the glucose levels in these mixtures over a wide range, it was found possible not only to reproduce most of the curves observed in vivo, but also to establish many of the theoretical aspects presented for these systems earlier (Kleinberg, I. J. dent Res. In press).

This study is now complete and in preparation for publication.

4. A study has also been carried out on the effect of different levels of some of the more common sugars (namely glucose, galactose, fructose, maltose, lactose and sucrose) on the rate of acid production by the organisms present in saliva sediment.

Certain "starchy" food stuffs which had been or have yet to be tested in vivo, were also included and these were, unrefined and refined wheat flour, white and wholemeal bread, and cooked and uncooked rice.

The findings with respect to the sugars were (i) that the rates of acid production with each of glucose, fructose, maltose and sucrose were essentially similar but differed from galactose and lactose both of which gave lower rates; (ii) galactose gave the slower rate of acid production for the latter than with the rate for lactose mid-way between that for galactose and that for the other sugars; (iii) the dissacharides gave the same results as their monosacharides equivalents, which clearly indicates that hydrolysis by the dissaccharidases present in saliva, which are chiefly of bacterial origin, must be very rapid. The decreased rate of acid production with lactose must be due to the presence of galactose which is produced when this sugar is hydrolyzed.

The polysaccharides mentioned above were compared to 5% glucose, which was used as reference. The acid production rates with each were very similar, with the results showing good agreement with those found in previous in vivo experiments in which white bread, white flour, cooked and uncooked rice gave the same pH fall as did 5% glucose. A comparison in vivo between 5% glucose, unrefined flour and wholemeal bread has yet to be made.

The study reported previously (see 1960 report) on the effect of various combinations of different glucose and urea concentrations on the rate of acid production in saliva has been expanded to include systems with higher concentrations of cells, since these conditions would, as found in the studies reported in 2 and 3 above, simulate more closely those found in plaque. The results observed were essentially similar to that for saliva in which the cell concentration is low. This study is now complete and is being prepared for publication.

A study similar to 3 above using urea instead of glucose as substrate is being carried out. It appears from the data thus far that the in vivo pH curves with this substrate (see 1960 report) can also be reproduced in vitro.

7. A study on the rate at which certain nitrogenous substrates other than urea can be broken down by the oral microorganisms to produce base is also in progress. Samples of bovine gingivae tissue as well as some bovine muscle tissue have each been homogenized and tested for possible effects on alkali production in saliva-sediment in vitro and in dental plaque in vivo. A slight pH fall was observed initially in the former system followed by a small pH rise which occurred after 48 hours. In the experiments vivo, when these homogenates were added continuously by syringe to selected dental plaques for a 30 minute period, no effect on plaque pH levels was observed. In viro tests for longer periods have yet to be carried out. Samples of human gingiva removed during gingivectomy gave similar results to bovine gingival tissue when both were tested in vitro. It should be pointed out here that, since these substrates cannot be broken down rapidly by the oral microorganisms, it would seem therefore that these substrates could not account for the previously made observation (Kleinberg, I. Biochem. J. 66, 61P, 1957) that fasting plaque pH levels are on the average approximately 1 pH unit more alkaline than the saliva washing them.

Another possible substrate which might be available to the oral bacteria for alkali production is the mucoprotein found normally in saliva. In experiments thus far, it has been found that with this substrate, the pH falls initially fairly rapidly and then requires from 24 to 48 hours to return to zero hour pH levels. Probably what is occurring is that the carbohydrate portion of the mucoprotein molecule is broken down fairly easily at first, giving the observed rapid initial pH fall, then when the carbohydrate portion is more or less exhausted, the alkali, which is slowly formed from protein breakdown, has time to accumulate and give the observed pH rise.

From the above studies, the present picture seems to be that the urea in saliva is the most likely substrate responsible for fasting plaque pH levels being above that for saliva. From the data in the vivo studies on the effect of different levels of urea on plaque pH in vivo (see 1960 report) and from the figures given by Nikiforuk et al (J. Ped., 49, 425, 1956) for urea levels in saliva, it seems that the salivary levels for this substrate could quite easily

account for this effect. Since elevated urea levels in saliva are associated with elevated levels in blood, it is conceivable that the protein in the diet could indirectly through the blood and saliva urea levels be supplying sufficient of this substrate to the oral bacteria to significantly affect the metabolism and possibly the composition of the oral flora.

An effort has been made to develop suitable ultramicro-analytical techniques which would permit chemical analysis of plaque material for the various constituents which may be affected by plaque pH changes resulting from changes in the metabolism of the plaque bacteria. The construction of suitable ultramicro-glassware has been greatly facilitated by the construction of a very simple ultramicro-burner. Also, since it has been found from preliminary studies on the solubility of plaque, that under alkaline conditions plaque will "dis-integrate", therefore the simple addition of NaOH has been used successfully to dissolve and disperse this material so that uniform sampling for analysis could be made.

At present, it is possible to conveniently estimate 0.02 μg samples of phosphorus, 0.5 μg . samples of calcium and 0.5 μg samples of magnesium with an error of less than 5%. It is intended to adapt other more macro-methods to similar levels so that together with the above, changes in plaque composition resulting from changes in bacterial metabolism and in certain properties of saliva can be elucidated.

For example, in a few preliminary experiments to date, it has been possible to show that when 5% glucose is supplied continuously to selected plaques in situ for a one hour period, along with the pH fall, the level of calcium in the plaque also falls, a finding which if confirmed would provide almost conclusive proof for Miller's Acid Decalcification Theory.

The solids content of plaque has been determined and found to be approximately 17%, the water content therefore, is approximately 83%.

Project II

Further studies on the effect of substances removed during the refining of carbohydrates on calcium phosphate solubility.

1. The study on the effect of pH on the solubility of calcium, phosphate and organic material in saliva has been continued and the tentative findings reported previously (see 1960 report) have been confirmed. In addition, it has been possible to show that when saliva supernatant (i.e. whole saliva minus the sediment formed after centrifugation at 3,000 r.p.m. for 15 minutes) is incubated at 37°C., different amounts of calcium, phosphate and organic material progressively precipitate out of solution with the result that after 24 hours little or no calcium is left, most but not all of the phosphate is still present while approximately one half of the organic material still remains. These findings provide support for the hypothesis that saliva, as it enters the oral cavity from the salivary ducts, undergoes certain changes (the most important seem to be CO₂ loss and exposure to numerous bacterial enzymes) which tend to result in the spontaneous precipitation of calcium from saliva, partly as calcium phosphate and partly as calcium bound to organic material, most probably mucoprotein. Since this precipitated organic material has the appearance of materia alba, it is intended by the techniques reported in 8 above to attempt to correlate the formation of materia alba in vivo to these experimental observations in vitro.
2. In preparation for extensive studies which have been planned to determine the relative solubilities of the sodium, potassium, magnesium and calcium salts of phytic acid and their effect on calcium

phosphate solubility, a large quantity of this acid has been carefully purified to serve as a stock material from which the various salts can be prepared. By means of titration curves, it has been possible, at least qualitatively, to show that the replacement of the hydrogen ions on the phytate molecule with either Na or K or both, results in the formation of soluble phytate salts whereas replacement with Mg or Ca produces salts of lesser solubility; this decreasing effect on solubility was greater with the latter ion (cf. McCance, R. A. and Widdowson, E. M. J. *Physiol.* 101, 44, 1942). The data from these curves have also given a good indication of which of the phytate salts will tend to increase or decrease the solubility of calcium phosphate.

Experiments are still in progress to study these effects quantitatively and to determine the mechanism by which phytate exerts its effects. Experiments are also in progress to determine the relative ease with which the various phytate salts can be hydrolyzed by the enzyme phytase to give the vitamin, inositol and a corresponding phosphate salt.

It is hoped from the above studies on phytic acid to test the grantee's present hypothesis that the removal of this substance during the refining of flour or the suppression of its solubility by the addition of CaCO_3 to the flour (McCance, R. A. and Widdowson, E. M. J. *Physiol.* 101, 44, 1942) may be an undesirable procedure. Although this addition of CaCO_3 may result in increased Ca uptake in the intestinal tract, on the other hand it is conceivable that phosphate absorption may be suppressed (i) by the mass action effect of the added calcium and (ii) by phytate solubility being

reduced to such an extent that the phytases that may be present, cannot act. With the recent findings in animal experiments that inorganic phosphate can result in a marked reduction in the caries incidence, this reduced availability of inorganic phosphate from cereals may be extremely important.

Principal Investigator: Dr. C. P. Leitch

Project No.: DA 31

Amount of grant: \$1,000.00

SUMMARY OF WORK

The dental research carried out here in 1960 has progressed along three main lines:

1. A new series of experiments using calcium⁴⁵ have been carried out on the availability of mineral components in the diets of the rat (as the results reported last year were felt to require confirmation). The new data, summarized in the attached table 1, show no change in the calcium concentration of the diet (as calculated on a weight basis) but a marked increase in the calcium content of the diet (as calculated on a weight basis) after injection. Thus, the calcium taken up is stored in a permanent manner.

2. The work on the utilization of dentin collagen by odontoblasts was continued using glycine-¹⁴C and proline-¹⁴C, with the same results as with glycine-¹⁴C, that is, the collagen - or pro-collagen - is synthesized in the cell and rapidly deposited to the outside as predentin matrix (see manuscript no. 2, enclosed).

3. Structures which are believed to be a new cell organelle - referred to as cell wall - have been described by one group in a number of cells. The observations have now been extended to the cytoplasm of odontoblasts and ameloblasts (see manuscript no. 3, enclosed).

APPENDIX E

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

January 1961

Subject: Entry of carbohydrates and proteins into teeth as shown with the help of radioactive elements

Grantee: Dr. C.P. Leblond

Project No.: DA 23

Amount of grant: \$4,400.00

SUMMARY OF WORK

The dental research carried out here in 1960 has progressed along three main lines:

1. A new series of experiments using calcium⁴⁵ have been carried out on the stability of mineral components in the dentin of the rat (as the results reported last year were felt to require confirmation). The new data, summarized in the attached table 1, show no change in the maximum concentration of calcium⁴⁵ in dentin (expressed as number of silver grains per unit area) at 1, 3, 6 or 9 days after injection. Hence, the calcium taken up is stored in a permanent manner.
2. The work on the elaboration of dentinal collagen by odontoblasts was continued using glycine-C¹⁴ and proline-C¹⁴, with the same results as with glycine-H³, that is, the collagen - or pre-collagen - is synthesized in the cell and rapidly deposited to the outside as predentinal matrix (see manuscript no. 2, enclosed).
3. Structures which are believed to be a new cell organelle - referred to as cell web - have been described by our group in a number of cells. The observations have now been extended to the cytoplasm of odontoblasts and ameloblasts (see manuscript no. 3, enclosed).

Table 1

OBSERVATIONS CONCERNING THE STABILITY

OF THE MINERAL COMPONENT (CALCIUM) OF DENTIN IN THE RAT

New set of data prepared in collaboration with Mr. G. Weinlander

(see attached)

The results obtained after a single injection of radiocalcium to 3-day old

rats sacrificed from 1-9 days later showed that, once radiocalcium is incorporated in dentin, its maximum concentration does not change with time.

This may be seen in the enclosed table in which the growth of dentin with age is shown in column 5, but the mean number of silver grains per unit area remains unchanged, as shown in the last column of the table. Hence, the calcium taken up in the tooth is stable in spite of the rapid growth of dentin.

MAXIMAL UPTAKE OF RADIOCALCIUM BY DENTIN AT
VARIOUS TIMES AFTER A SINGLE INJECTION
TO 3-DAY OLD RATS

Days after injection	Animal	Body weight at sacrifice (gm)	Jaw {u=upper l=lower}	Minimal width of dentin in area examined (μ)	Peak ⁴⁵ content	
					Distance from outer edge of dentin (μ)	Mean number of graining per 34 μ^2
1	A1	9.5	u	35	26	12.2
	B1	9.5	u	46	38	8.0
			l	52	38	12.4
	C1	10.0	u	23	20	8.0
	D1	9.0	u	41	26	11.5
	D2	10.5	u	46	38	11.7
	Mean	9.7		40.5	31.0	10.6
3	A1	13.0	u	46	9	8.7
			l	64	26	13.3
	B1	11.5	u	75	26	12.7
			l	70	26	13.0
	C1	13.5	u	87	26	12.8
			l	64	20	9.5
	C2	11.5	l	70	32	10.8
	Mean	12.4		68.0	23.6	11.5
6	A1	17.5	u	122	23	8.4
	A2	16.0	u	139	26	9.7
			l	122	26	7.5
	B1	16.5	u	197	38	13.9
	C1	16.0	u	139	26	14.8
			l	128	20	11.8
	D1	16.5	u	157	20	11.7
			l	174	38	12.0
	Mean	16.5		147.3	27.1	11.2
9	A2	25.0	u	197	20	10.1
			l	244	20	11.2
	B1	22.0	u	220	26	8.3
	C1	25.0	u	168	15	12.5
			l	220	20	12.1
	D1	25.0	l	203	49	9.1
	Mean	24.3		208.7	25.0	10.6

(Manuscript No. 2)

(Accepted for publication in the Proceedings
of the 1st International Congress of Histo-
chemistry and Cytochemistry)

ELABORATION OF DENTINAL COLLAGEN IN ODONTOBLASTS
AS SHOWN BY RADIOAUTOGRAPHY AFTER INJECTION OF
LABELLED GLYCINE AND PROLINE

C.P. Leblond

Department of Anatomy, McGill University

The role of fibroblasts in producing collagen fibers has long been a controversial problem. Cameron who reviewed the subject in 1952 listed a series of authors who claimed that the fibers are elaborated in the cells. To this list should be added the name of Wassermann who observed with the electron microscope that collagen filaments arose within the cytoplasm next to the cell membrane (1954). Another school of thought was that the cells secrete a diffusible enzyme which, at some distance, induces soluble tissue protein to precipitate as collagen fibers (Nageotte, 1931). Similarly, the recent electron microscope investigations of Porter and Pappas (1959) demonstrated that filaments with collagen periodicity appeared outside rather than inside the cells, but in close association with the cell membrane.

The origin of collagen might be investigated with odontoblasts more readily than with fibroblasts. Not only are odontoblasts readily found between the pulp and dentin of teeth, but they are oriented cells, with an apex bearing the odontoblastic process (Tomes fiber) on the dentinal side. Furthermore, in growing teeth, radioautography of mice given C^{14} -labelled bicarbonate or glucose showed a rapid and continuous deposition of matrix next to the odontoblasts (Greulich and Leblond, 1954; Kumamoto and Leblond, 1958) and this matrix contains nearly 95% collagen (Stack, 1955). It thus appears that odontoblasts, like fibroblasts, may play a role in collagen formation. Using the electron microscope, Watson and Avery (1954) saw small collagen filaments adjacent to, but not within the odontoblasts. They suggested that collagen fibers are formed on the wall of these cells and gradually move away while increasing in size and number. These various results made it likely that the odontoblasts of growing teeth would be suitable for an investigation of collagen formation.

Collagen has an amino-acid composition different from that of most proteins, with glycine making up 32.5%, proline 12.2%, and hydroxyproline 9.8% of the amino-acid residues in the molecule (Bowes and Kenten, 1948). Free glycine appears to be the precursor of the glycine residues

(Neuberger et al, 1951) and free proline, of both proline and hydroxyproline residues (Stetten, 1949). It was, therefore, decided to examine radio-autographically the behavior of these two precursors in relation to the odontoblasts of growing teeth in the hope of obtaining information on collagen production.

Two series of experiments were carried out. In the first series, the behavior of glycine labelled with tritium was examined in the continuously growing incisor teeth of adult male mice sacrificed from 30 minutes to about 1 month after a single injection (Carneiro and Leblond, 1959). A second series was designed to complete this investigation in three ways. First, 3-day old rats and adult male mice were sacrificed at very early intervals (5 and 15 minutes) after injection of tritium-labelled glycine. Secondly, similar animals were sacrificed at various time intervals after administration of glycine labelled with radio-carbon instead of tritium. Finally, similar animals were given proline- C^{14} and treated in the same manner.

The odontoblasts of the continuously growing incisor teeth were examined in the adult male mice, and those of molar and incisor teeth in 3-day old rats. The results were substantially the same in the odontoblasts of any one of these growing teeth.

The observations made in the two series of experiments will be considered in the presentation of the results.

Behaviour of glycine- H^3

As early as 5 minutes after injection of methyl-labelled glycine to adult mice, a discrete radioautographic reaction was seen over the odontoblasts, but none over the surrounding spaces. At 15 minutes after injection, the pattern was the same but the reactions over the odontoblasts were more intense. Abundant silver grains were over the cytoplasm, with the exception of the apical region and its odontoblastic process, over which grains were rare. Only the occasional grain was seen over the nucleus. It was earlier thought that in odontoblasts the reaction was restricted to a Golgi-like zone (Carneiro and Leblond, 1959). However, the better preparations now available made it clear that the radioactive zone extended to most of the cytoplasm.

At 30 minutes after injection, the same cytoplasmic distribution of the reactions was seen, but, in addition, a small but significant accumulation of grains appeared outside the apex of the cell on the predentin side (fig. 1). The intense reaction over the odontoblasts contrasted with the slight reaction present over the pulp cells.

Four hours after injection, the odontoblasts had lost nearly all their radioactive material and they exhibited a slight reaction comparable to that seen over pulp cells; but an intense reaction band was now seen over

predentin close to the odontoblasts (fig. 2). By 35 hours, an equally intense reaction band was visible over dentin itself not far from predentin, which at that time showed little or no radioactivity (fig. 3). At 7 days only traces of radioactivity were present over all cell types (odontoblasts, ameloblasts and pulp cells), but the reaction band of dentin had not changed in intensity. This band was at some distance from the odontoblasts, as a considerable amount of new, non-radioactive dentin had been added in-between (fig. 4).

For the interpretation of the results, it may first be recalled that, in the course of histological fixation and processing, most small molecules are washed out of the tissues, but substances with a large molecular weight, such as some proteins, nucleic acids, etc., are retained in the sections. Now it was observed that the reactions varied greatly in intensity with various amino-acids (compare figs. 4 and 5), but the pattern was the same after injection of methionine- H^3 or leucine- H^3 (fig. 5) as with glycine- H^3 (fig. 4), so that these three amino-acids seemed to have contributed to the formation of the same glycine-rich, leucine- and methionine-poor protein(s). The behaviour of this protein is indicated by the radioautographic sequence of events. The cytoplasmic reactions seen 5-30 minutes after injection (fig. 1) indicated that the protein is elaborated within the odontoblasts. The rapid waning of the cellular reactions, while intense ones appeared next to the cell apex in predentin (fig. 2), indicated that the protein is promptly released from the cell apex into predentin. Since later the reaction was seen in dentin itself without change in intensity (compare figs. 3 and 4) the protein under study was not visibly influenced by the transformation of predentin into dentin.

Behaviour of glycine- C^{14}

Since the glycine- H^3 used in the first experiment was labelled only in the methyl group, there was a possibility that only the behaviour of this group was being traced. This possibility was examined by using two other types of labelled glycine, one uniformly labelled with tritium, and the other with radio-carbon. Particularly interesting was the use of the latter, glycine- C^{14} , since this substance made it possible to check whether the tritium label exchanged with other hydrogen atoms or behaved like the carbon atoms to which it is attached. In any event, if the three types of labelled glycine were to yield the same radioautographic distribution, it could be concluded that the results in figures 1-4 show the behaviour of the whole molecule.

The radioautographs, although less sharp with glycine- C^{14} than glycine- H^3 , showed the same pattern of distribution, with a reaction over the cytoplasm of odontoblasts at 30 minutes (fig. 6), over predentin at 4 hours after injection (fig. 7) and over dentin at later time intervals.

It is, therefore, concluded that a glycine-rich protein is built up by odontoblasts and released to predentin.

Behaviour of proline

The high content of collagen in dentin (Stack, 1955) and the finding of collagen filaments next to odontoblasts (Watson and Avery, 1954) strongly suggested that collagen might be the protein involved in our experiments. Since proline appears to be the precursor not only of the proline and hydroxyproline, but also of some glutamic and aspartic acid in collagen (Smith and Jackson, 1957), it may be estimated that proline is the precursor of some 25% of the collagen amino-acids (and glycine of some 32.5%). Hence, if collagen were to be synthesized by odontoblasts, not only should labelled proline be taken by these cells, but the reaction should be of the same order of intensity as with labelled glycine. This was found to be the case.

The results yielded again a pattern of uptake in odontoblasts at 30 minutes (fig. 8) and release to predentin at 4 hours (fig. 9). The reactions were comparable to those produced by glycine (figs. 6-7), but far more intense than those due to other amino-acids.

It is concluded that all the evidence available, particularly the high uptake of glycine (figs. 1-7) and proline (figs. 8 and 9), in contrast with a low uptake of methionine and leucine (fig. 5) indicated that the protein formed in the cytoplasm of odontoblasts and promptly released to predentin consists of collagen.

Formation of collagen

Schmitt, Gross and Highberger (1954) suggested that rod-like elements characterized by a molecular weight of 300,000 and referred to as "tropocollagen" aggregate to make up collagen filaments. The same authors later speculated that tropocollagen or some other monomer was synthesized within fibroblasts and made up into filaments outside the cells (Gross et al, 1955). Support for this opinion was derived from observations of Smith and Jackson (1957) who, on addition of proline- C^{14} to a fresh suspension of osteoblasts, detected protein-bound hydroxy-proline- C^{14} twenty-four hours before banded filaments became visible with the electron microscope. The hydroxy-proline-containing precursor would be a non-banded structure which would transform into collagen fibers without change in hydroxyproline content (Jackson and Smith, 1957). In any event, the results of these authors demonstrate that collagen formation is a two-step affair.

So do the present results. The odontoblast cytoplasm would elaborate a collagen precursor rich in glycine and proline like collagen itself (figs. 1 and 8). However, the cytoplasm does not show banded filaments (Watson and Avery, 1954) and, therefore, the intracellular collagen precursor must have a structure (unbanded, small-size) allowing it to escape detection

with the electron microscope. Since this would also be the case with the monomers postulated by Gross et al (1955) or the hydroxyproline-containing precursor of Jackson and Smith (1957), it may be that these and our intracellular collagen precursor are one and the same thing.

However, soon after the formation in the cell, the collagen precursor is secreted from the apical end of the odontoblast to become part of predentin. At 4 hours, the silver grains are seen throughout predentin, with predominance close to the odontoblasts (fig. 2). This observation is consistent with Watson and Avery's (1954) conclusion that new collagen filaments (100 Å diameter) appear next to the odontoblasts and these thicken up to 600 Å in diameter as they are pushed away. The grains located next to the odontoblasts would correspond mainly to the collagen precursor molecules lining up to form new filaments, while the grains farther away (fig. 2) would be due to molecules added to the surface of filaments so as to increase their diameter.

Incidentally, sites of bone formation showed a comparable sequence of events. Thus, after injection of labelled glycine (Carneiro and Leblond, 1959) or proline, a radioautographic reaction was observed throughout the cytoplasm of osteoblasts at 5-30 minutes and next to the apex of these cells in the prebone (osteoid) at 4 hours. Hence, in osteoblasts as in odontoblasts, a precursor of collagen is elaborated in the cytoplasm and soon thereafter released to the prebone.

Summary

It is concluded that a collagen precursor is elaborated in the cytoplasm of the odontoblasts of growing teeth and promptly deposited outside these cells to become the collagen fibers of predentin. Similar results obtained with osteoblasts suggest that the two steps observed (cellular elaboration of a collagen precursor and its release outside the cell as collagen fibers) may exist in all sites of collagen formation.

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LEGEND OF FIGURES

Figures 1-3 Radioautographs of H. E. stained section of growing incisor teeth from adult male mice injected with 5 uc of glycine-2- H^3 (13 mc/mM) per gram of body weight and sacrificed after thirty minutes (fig. 1), four hours (fig. 2) or thirty-five hours (fig. 3). From left to right note pulp, P; odontoblasts, O; predentin, Pd; dentin, D; enamel, E; and ameloblasts, A. x 225

Strongly radioactive material (horizontal arrows) is seen in the cytoplasm over odontoblasts at 30 minutes (fig. 1) in predentin at 4 hours (fig. 2), and in dentin at 35 hours (fig. 3).

(In any area, the distance between the reaction band and the dentino-enamel junction is an index of how thick dentin was at the time of injection in the area used for photography).

Figure 4 Radioautograph of H. E. stained section of growing incisor tooth from an adult male mouse sacrificed 7 days after injection of glycine- H^3 under the same conditions as in figures 1-3. x 225

The labelled dentinal matrix formed immediately after injection (horizontal arrow) is now located far from the odontoblasts.

Figure 5 Radioautograph of H. E. stained section of growing incisor tooth from an adult male mouse sacrificed 7 days after injection of 5 uc of DL-leucine-4,5- H^3 (150 mc/mM) per gram of body weight. x 225

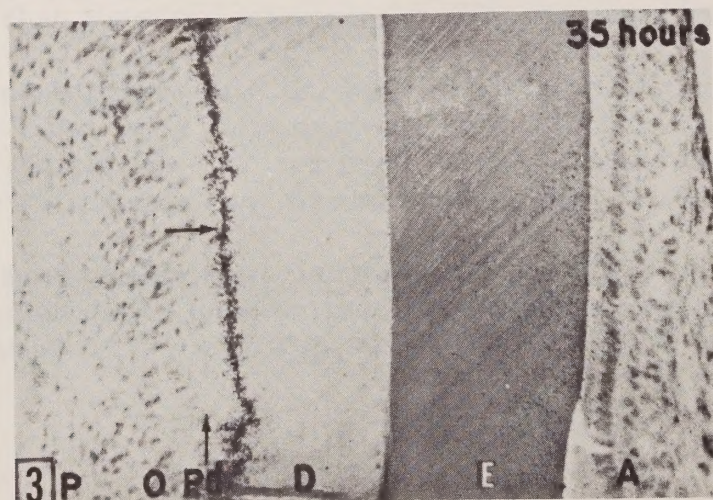
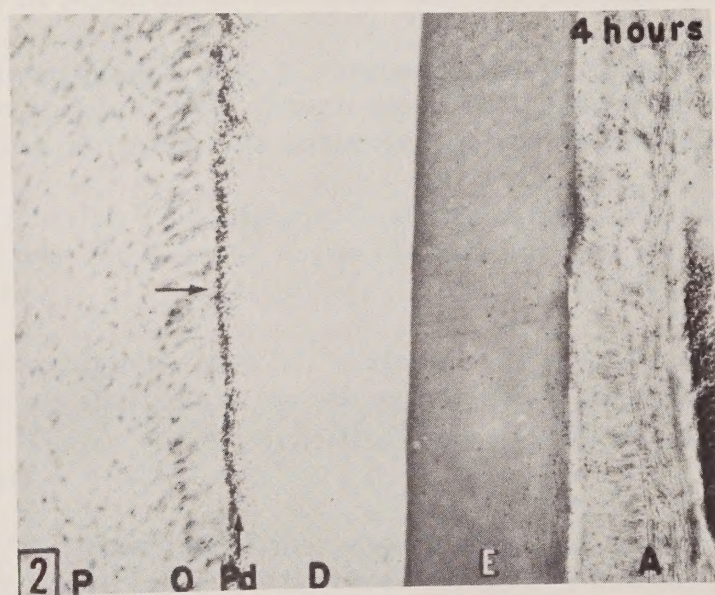
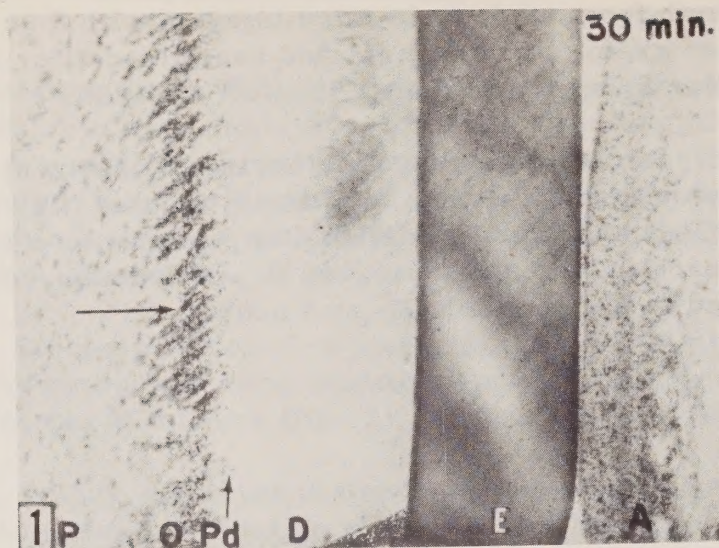
The reaction of the labelled dentinal matrix formed immediately after injection (horizontal arrow) is considerably weaker than that observed after glycine- H^3 injection (fig. 4).

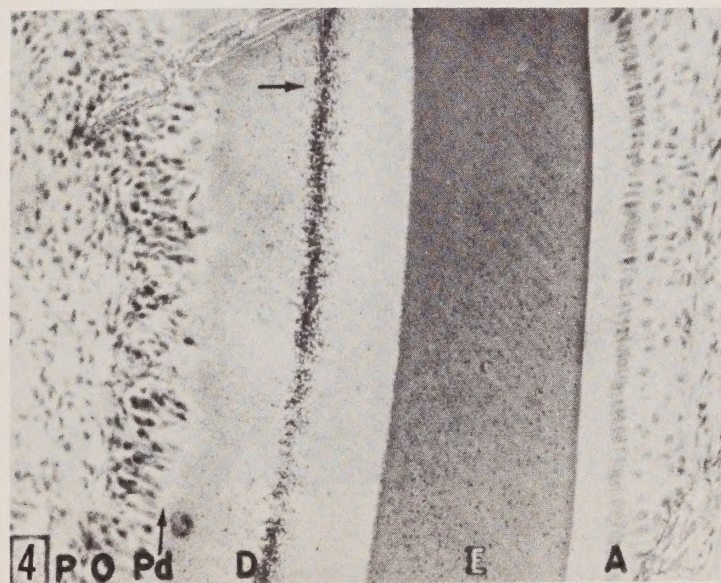
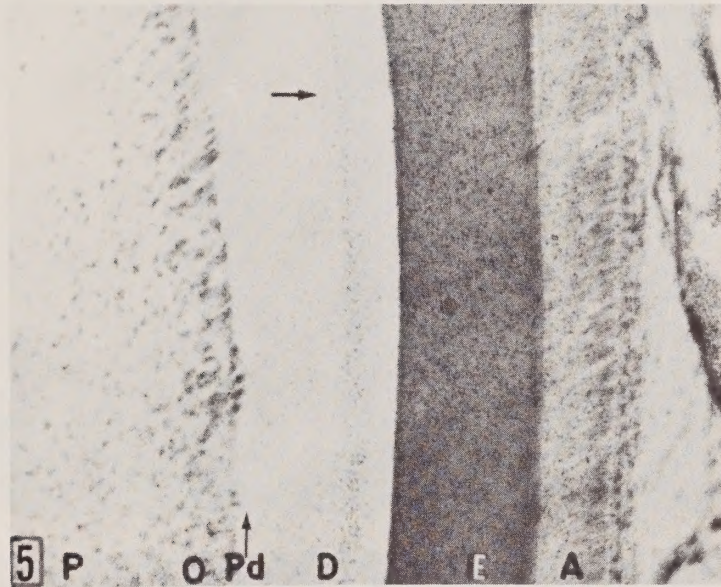
Figures 6-7 Radioautographs of H. E. stained sections of growing incisor teeth from adult male mice injected with 3 uc of uniformly labelled glycine- C^{14} per gram of body weight and sacrificed after 30 minutes (fig. 6) and 4 hours (fig. 7) after injection. x 225

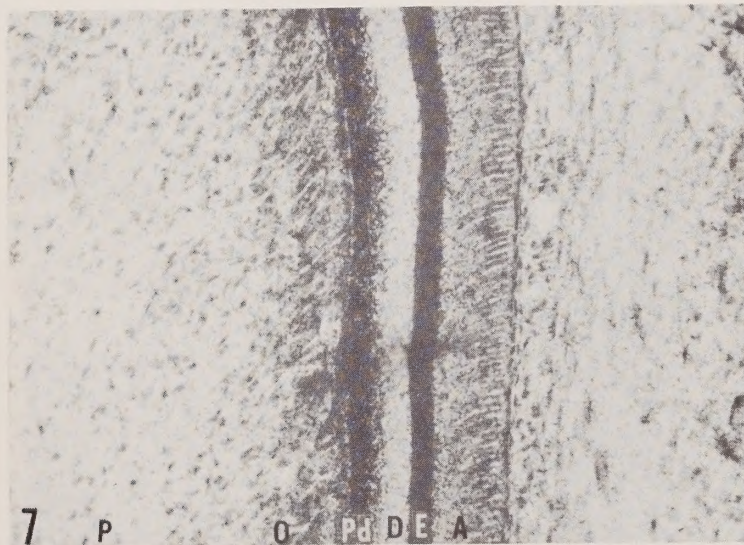
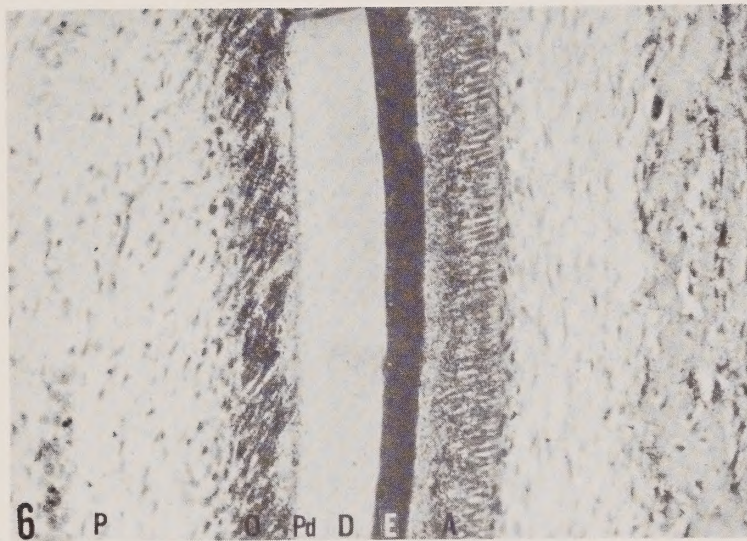
Radioactive material (horizontal arrows) is seen in the cytoplasm over odontoblasts at 30 minutes (fig. 6) and in predentin at 4 hours (fig. 7).

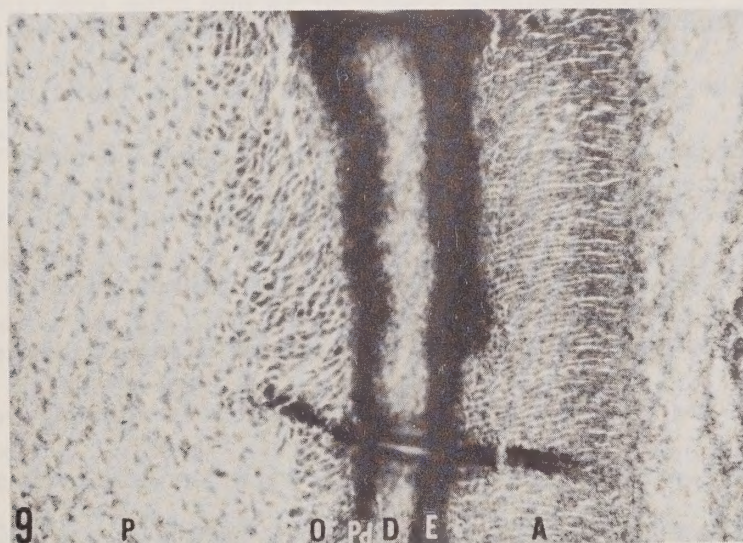
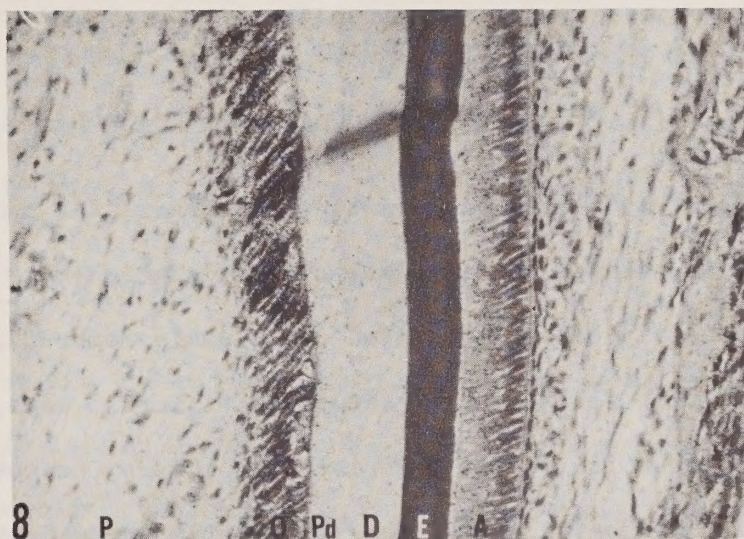
Figures 8-9 Radioautographs of H. E. stained sections of growing incisor teeth from adult male mice injected with 3 uc of proline- C^{14} per gram of body weight and sacrificed after 30 minutes (fig. 8) or 4 hours (fig. 9) after injection. x225

Radioactive material (horizontal arrows) is seen in the cytoplasm of odontoblasts at 30 minutes (fig. 8) and in predentin at 4 hours (fig. 9). (With this precursor, a rather strong reaction was also observed in ameloblasts, A, at 30 minutes and in pre-enamel, E, at 4 hours).









THE DESMOSOME-CELL WEB SYSTEM IN THE CELLS OF TOOTH BUDS IN 3 DAY OLDALBINO RATS

E. Kallenbach and Y. Clermont

Department of Anatomy

McGill University

In the epithelial cells lining the intestinal lumen, the ducts of glands, etc., there are fine intracytoplasmic fibrils which make up a web-like layer immediately below the apical cytoplasmic membrane. In other cell types, similar fibrils are arranged in different ways, since they make up structures which may be described as pillars, baskets, tubes, tonofibrils, etc. In all cases, the groups of fibrils were identified by a technique which included successive treatments with tannic acid, phosphomolybdic acid and the dye amidoblack (the so-called TPA technique). The network of TPA-stained fibrils in their various forms were described under the name "cell web" (Leblond, Puchtler and Clermont, '60). The TPA technique stains not only the cell web fibrils but also structures such as the terminal bars and the desmosomes which are considered as sites of attachment between adjacent cells. Actually the cell web fibrils converge on the desmosomes and terminal bars and as it was shown with the electron microscope appear to insert on the plasma membrane which is thickened at that point. The TPA stain observed at the level of terminal bars and desmosomes is probably due in part to cell web fibrils.

Upon casual observation it was found that the ameloblasts which initially derive from the epithelium of the oral mucosa (known to contain a cell web in the form of tonofibrils) as well as some of the odontoblasts which derive from the mesenchyme of the pulp do contain some TPA stained material.

In fact these elements were already shown to contain desmosome-like structures (Ansell, 1882; Cohn, 1897) and more recent electron microscope investigations confirmed these early cytological findings (Watson and Avery, '54; Reith, '60). The purpose of the present report is to describe the more detailed observations made on odontoblasts, ameloblasts, as well as on the surrounding elements seen in the various regions of tooth buds of rats in sections stained with the TPA technique.

Material and Results

Upper and lower jaws of 3-day old rats were removed and fixed in Carnoy; following dehydration through alcohol and methylbenzoate they were imbedded in paraffin. 3 μ sections were stained with tannic acid, phosphomolybdic acid and amido black according to the technique of Puchtler and Leblond ('58).

Odontoblasts

The odontoblasts seen at the level of the cervical loops (region I, fig. 1) do not contain any TPA stained material. Higher up, when the odontoblasts become well differentiated and start to produce dentin (region II, fig. 1), some TPA stained desmosomes become visible at the apical edges of the cells. At this point no TPA stained cell web material could be detected. Above this region when dentinogenesis is well underway (region III, fig. 1) the odontoblasts give rise to the dentinal (Tomes) fibres extending into the dentinal tubules. In these cells some TPA stained material (it is difficult to resolve fibrils in it) extends from the desmosomes into the dentinal fibres (figs. 2,8). This material would be

equivalent to the apical cell web seen in other epithelial cells. At the base (pulp side) of these cells no desmosome-cell web system could be found.

Incidentally, a pair of centricles were nicely demonstrated with the TPA method over the apical extremity of the odontoblast nuclei.

Ameloblasts

In the region of the cervical loop (region I, fig. 1) the cells of the inner enamel epithelium do not show any TPA-staining material. Higher up, when these cells form a distinct layer of columnar cells now referred to as ameloblasts, TPA stained desmosomes appear at the pole of the cell in contact with the intermediate layer, i.e. the pole, distal to the basement membrane. At first no cell web material could be seen connecting the desmosomes; at a later stage of development, however, some TPA stained material appears forming a thin layer between the desmosomes (fig. 3). Since these cells are in the process of being repolarized (Beams and King, '33), this pole will be referred to as a basal pole.

Farther up (region II, fig. 1), some TPA stained desmosomes appear at the other or apical pole of the ameloblasts, but no web is seen between desmosomes. But once the Tome's process had appeared, some TPA stained material was seen running from desmosome to desmosome, thus forming an apical web (fig. 4). Since, meanwhile, the basal web is retained, these mature ameloblasts show two desmosome-cell web systems at the apical and basal extremities of the cells respectively (fig. 9).

Farther up again (region III, fig. 1) once amelogenesis had started additional TPA stained fibrils appeared. These take the form of a bundle running axially from the apical to the basal web. At the level of the

nucleus, this bundle splits into smaller units which surround the nucleus. At the apical end this so-called axial web (fig. 5,9) gives off fibrils which reach the desmosomes (fig. 9).

Toward the tip of incisors some more of these axial web fibrils appear in ameloblasts and give to the cytoplasm a texture similar to that of prickly cells of the stratified squamous epithelium (which contain tonofibrils in the form of a dispersed cell web, Leblond, Puchtler and Clermont '60).

Cells of the intermediate layer

A typical intermediate layer appears at the borderline of regions I and II (fig. 1). It is composed of 2 or 3 layers of closely packed polyhedral cells. At the interfaces between these cells numerous delicate desmosomes could be seen (barely distinguishable in fig. 6,7). Although at the limit of visibility with the light microscope some delicate TPA stained filaments were seen joining these desmosomes which are arranged in more or less regular rows (fig. 9). These delicate fibrils would thus form a cell web of the circumferential type (see Leblond, Puchtler and Clermont, '60 enclosed) connected to an array of desmosomes which hold the cells close together.

Cells of the stellate reticulum and outer enamel epithelium

These irregular stellate shaped cells contain one or more bundles of cell web fibrils (fig. 6). No desmosomes could be observed at the junction between these elements.

COMMENTS

A desmosome-cell web system as outlined by the TPA technique was

found to be present in odontoblasts and ameloblasts but showed various degrees of organisation depending on the state of development of the cells. Thus at the level of the cervical loops elements not yet differentiated into odontoblasts or ameloblasts did not show desmosomes or cell web. Then as the cells became well differentiated and assumed their characteristic prismatic shapes desmosomes and cell web appeared. Such structures were present in all cells having constant shapes (Leblond, Puchtler and Clermont, '60).

It is interesting to note that the formation of desmosomes, i.e. of points of attachment between cells, always preceded the appearance of the cell web, suggesting that the formation and growth of the cell web fibrils may be initiated by or originate within the desmosomal structures themselves. Furthermore in the ameloblasts the basal web appeared first and later, probably following the repolarization of the cells demonstrated by Beams and King ('33), the apical web was seen. The desmosomes, a series of which form the so-called terminal bars, have already been noticed by several authors in both odontoblasts (Ansell, 1886; Lehner and Plenk, '36; Watson and Avery, '54; etc.) and ameloblasts (Ansell, 1886; Cohn, 1897; Reichenback, '26, Reith, '60; etc.), but the presence of a delicate system of cell web fibrils connected to these desmosomes have generally been overlooked. Except maybe for the axial web fibrils of the ameloblasts which have been described as a "central fiber" by Lams ('20) and Held ('26). However, these authors have not seen connections between this central fiber with apical and or basal web and with desmosomes as shown in the present report.

It is interesting to note the presence of a cell web and/or desmosomes

LEGEND OF FIGURES

in the cells of the intermediate layer and in the stellate reticulum. This desmosome-cell web system may derive from the desmosomes cell web (tonofibrillar) structure observed in the stratified squamous epithelium of the oral mucosa which gives rise initially to these parts of the tooth bud.

The desmosome-cell web system of odontoblasts and ameloblasts may contribute to give to these elements their permanent prismatic shape necessary to the regular formation of enamel and dentin of the developing tooth.

Fig. 1 - Ameloblasts from region I showing a TPA stained desmosome-cell web system (B) at one pole of the cell. No similar system was seen at the other pole of the cell (A). An ameloblast in mitosis can be recognized (M).

Fig. 2 - Ameloblasts from regions II and III showing desmosome-cell web system at the poles of the cells. The so-called apical web (A) and basal web (B).

Fig. 3 - Ameloblasts from the top of region III (indicated) showing both apical (A) and basal (B) desmosome-cell webs and the axial web fibrils (AX) running from the apical to the basal cell.

Fig. 4 - Ameloblasts from region I showing a TPA stained desmosome-cell web system (B) at one pole of the cell. No similar system was seen at the other pole of the cell (A). An ameloblast in mitosis can be recognized (M).

Fig. 5 - Ameloblasts from regions II and III showing desmosome-cell web system at the poles of the cells. The so-called apical web (A) and basal web (B).

Fig. 6 - Ameloblasts from the top of region III (indicated) showing both apical (A) and basal (B) desmosome-cell webs and the axial web fibrils (AX) running from the apical to the basal cell.

Fig. 7 - Ameloblasts from region I showing a TPA stained desmosome-cell web system (B) at one pole of the cell. No similar system was seen at the other pole of the cell (A). An ameloblast in mitosis can be recognized (M).

Fig. 8 - Ameloblasts from regions II and III showing desmosome-cell web system at the poles of the cells. The so-called apical web (A) and basal web (B).

Fig. 9 - Ameloblasts from the top of region III (indicated) showing both apical (A) and basal (B) desmosome-cell webs and the axial web fibrils (AX) running from the apical to the basal cell.

Fig. 10 - Ameloblasts from region I showing a TPA stained desmosome-cell web system (B) at one pole of the cell. No similar system was seen at the other pole of the cell (A). An ameloblast in mitosis can be recognized (M).

LEGEND OF FIGURES

Figure 1 Diagram of a sagittal section through a molar tooth bud from a 3-day old rat. The various components are indicated. Region I corresponds to the area of the cervical loop where odontoblasts and ameloblasts differentiate; region II, to the area of early dentin formation; region III, to the area of both dentin and enamel formation.

Figures 2-7 Microphotographs of various cellular components of the tooth bud stained with the TPA technique. Magnification approximately 1200 times.

2 - Odontoblasts from region II. Desmosomes (D) can be seen at the apex of cells. Some cell web (W) material is seen between the desmosomes and going into the dentinal process seen in the dentinal tubule (arrow).

3 - Ameloblasts from region I showing a TPA stained desmosome-cell web system (B) at one pole of the cell. No similar system was seen at the outer pole of the cell (A). An ameloblast in mitosis can be recognized (M).

4 - Ameloblasts from regions II and III showing desmosome-cell web system at the poles of the cells, the so-called apical web (A) and basal web (B).

5 - Ameloblasts from the top of region III (incisor) showing both apical (A) and basal (B) desmosome-cell webs and the axial web fibrils (AX) running from the apical to the basal cell web.

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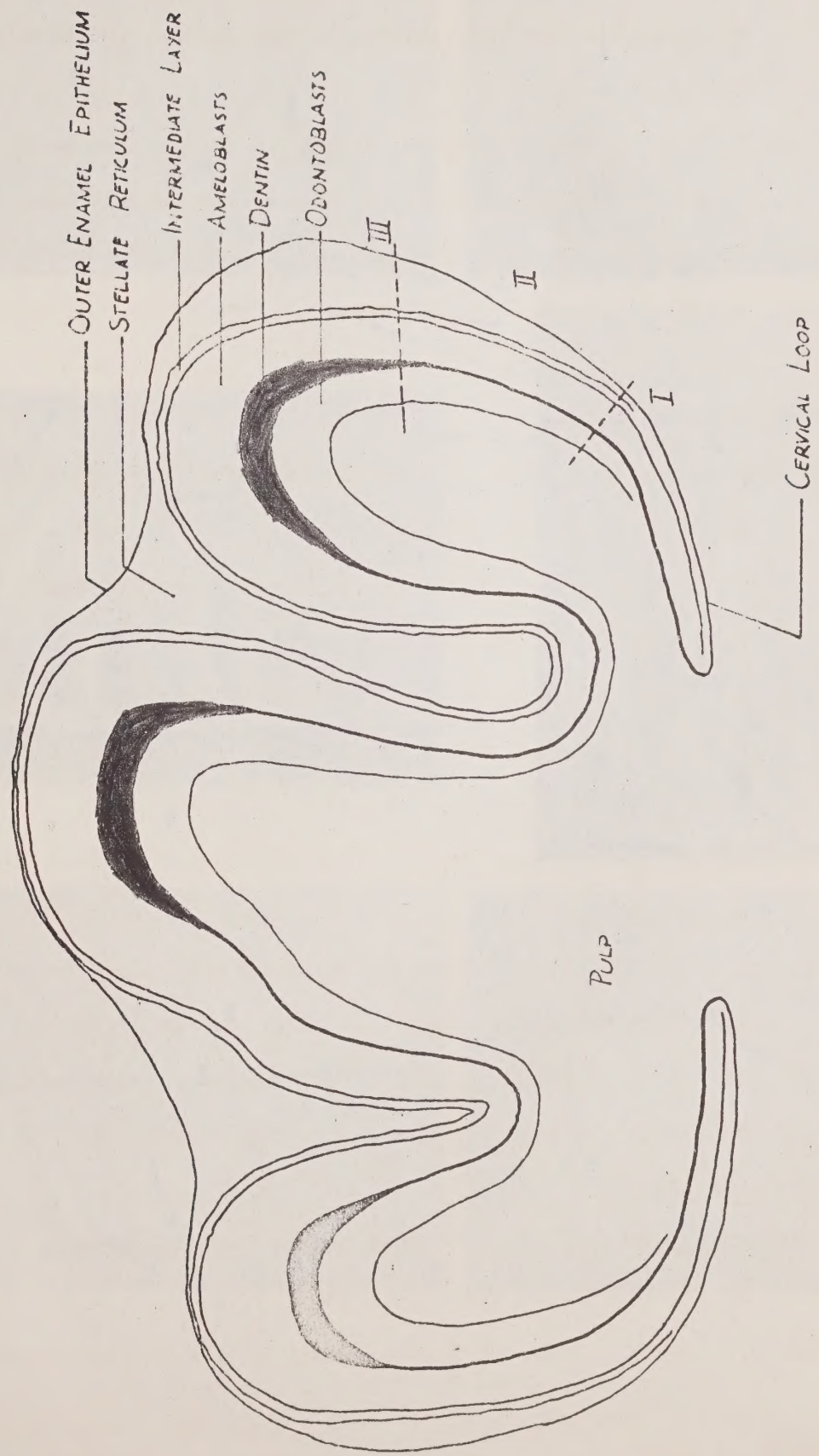
6 - Ameloblasts intermediate layer and stellate reticulum of region II showing the basal desmosome-cell web system of ameloblasts (B), the rows of desmosomes (D) seen at the interface between cells of the intermediate layer, and cell web fibrils (T) in cells of the stellate reticulum.

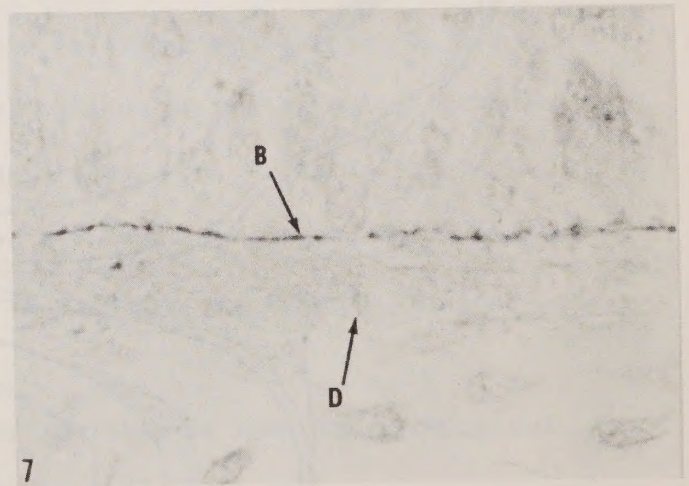
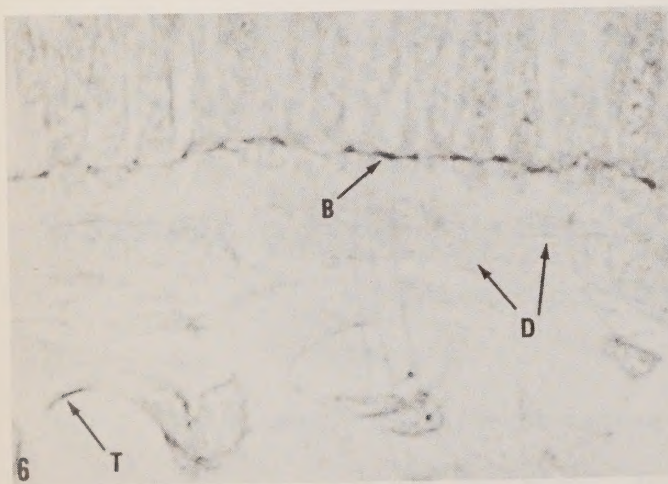
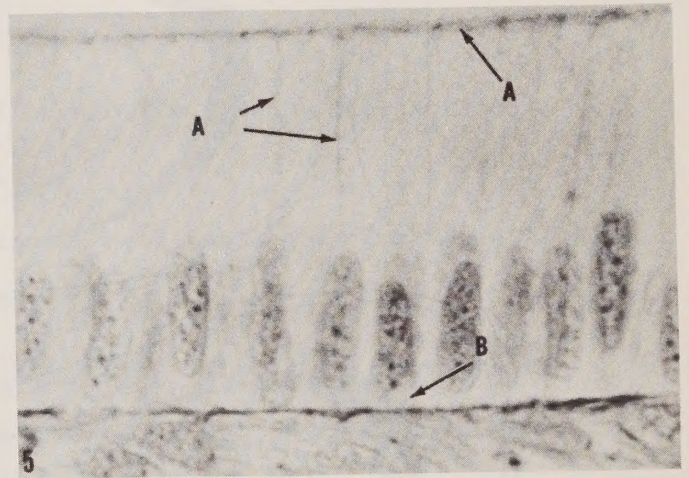
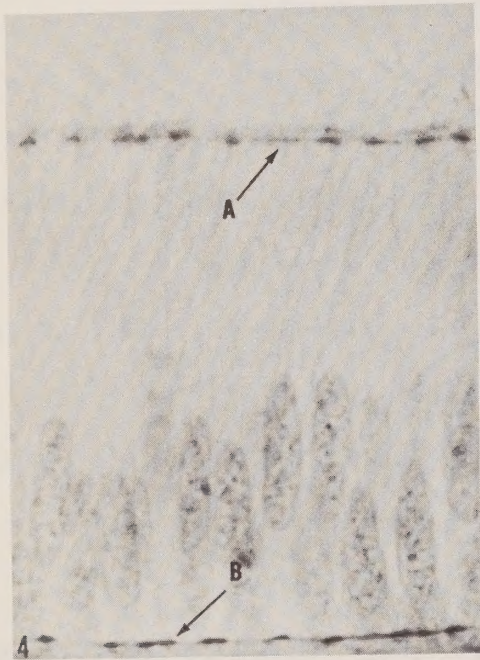
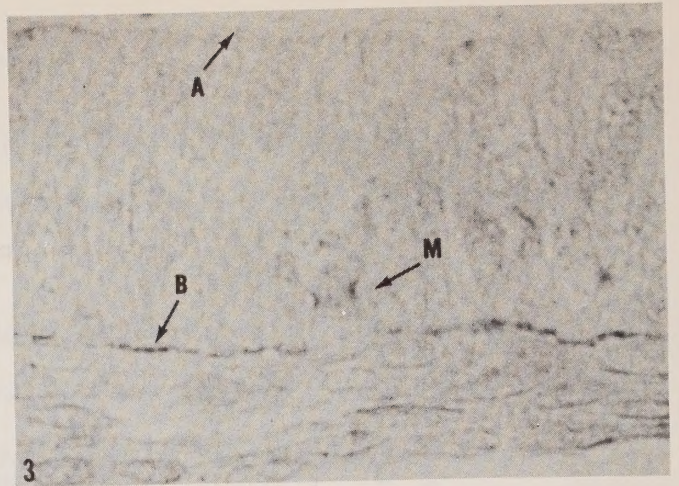
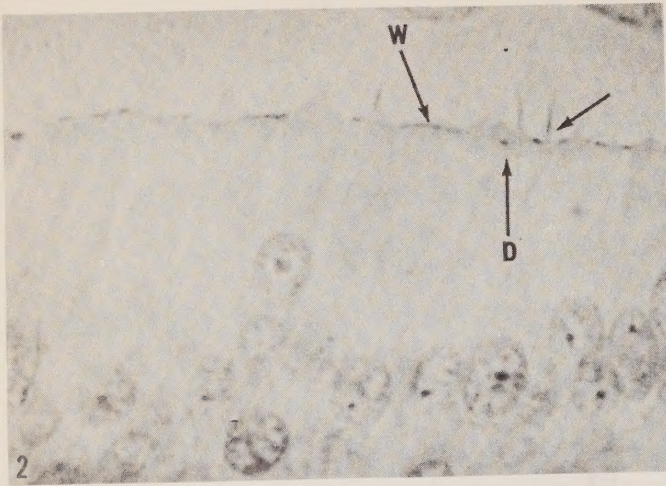
7 - Intermediate layer of region II showing delicate desmosomes (D) at the interfaces of polyhedral cells. The basal cell web of ameloblasts can also be seen (B).

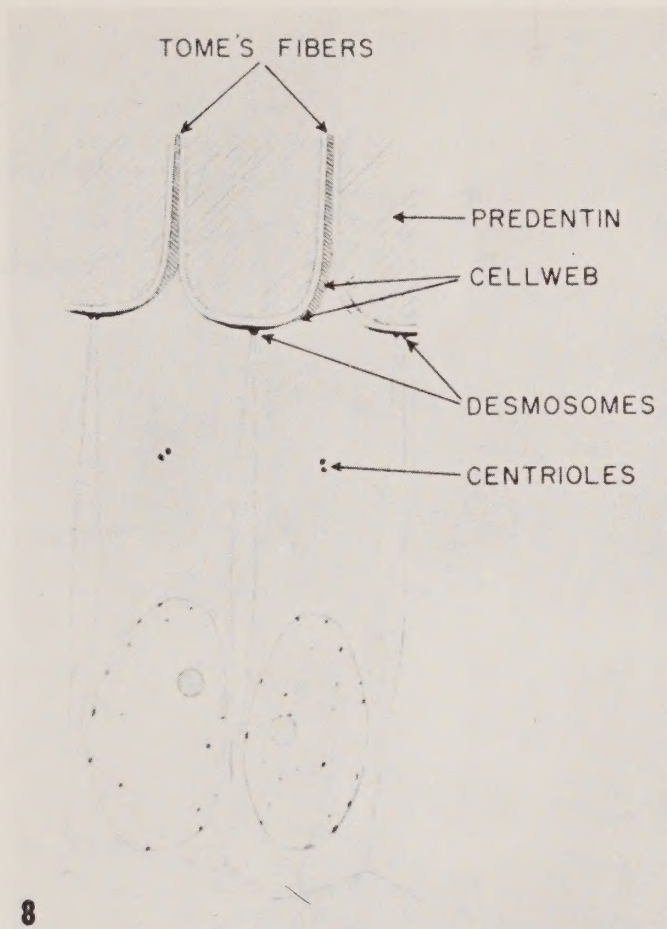
Figures 8 and 9 Schematic drawings summarizing observations made on TPA stained odontoblasts and ameloblasts.

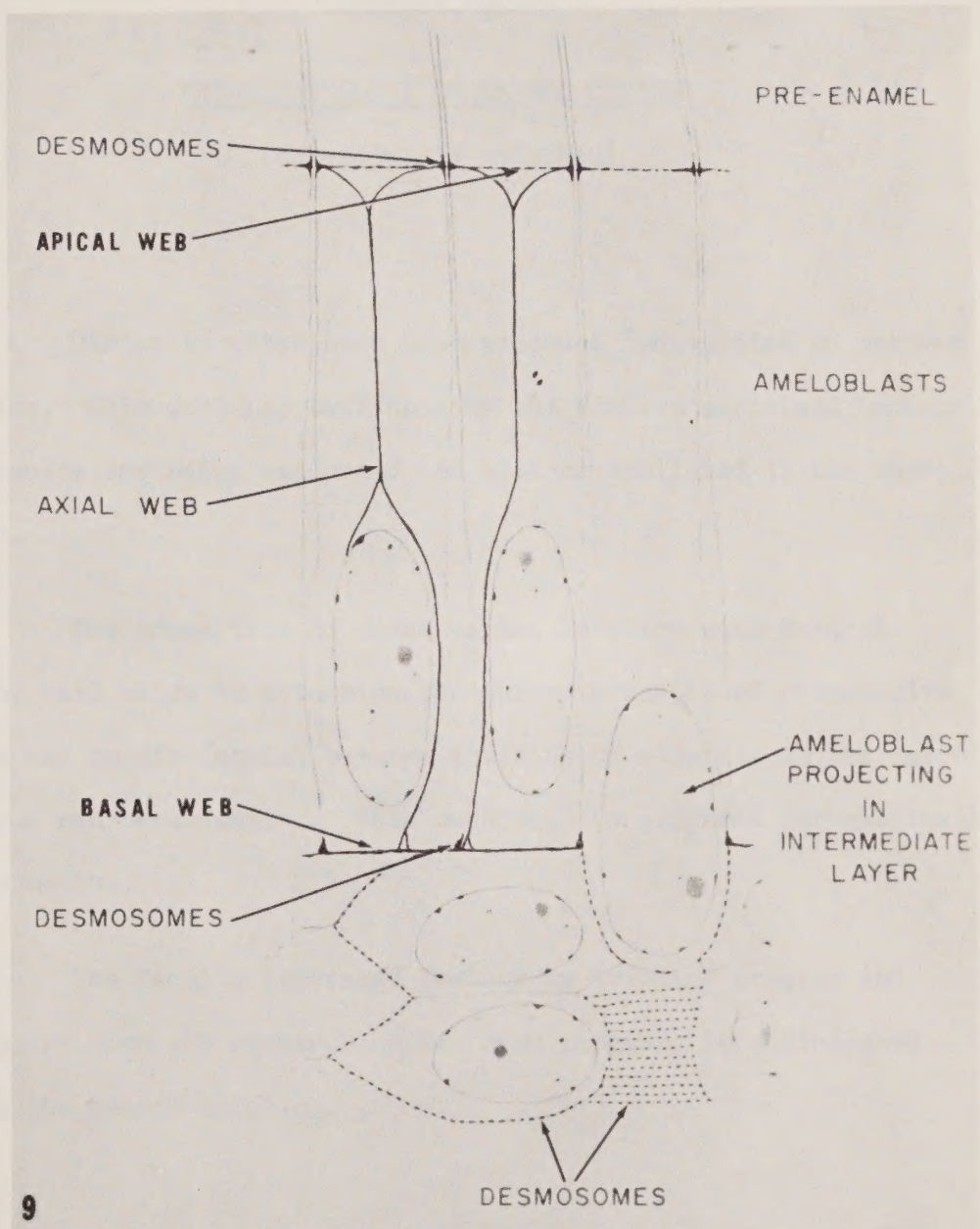
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APPENDIX F

DA-79

PROGRESS REPORT

"HISTOPATHOLOGY OF ENAMEL CARIES"

(a reprint is attached)

Caries in vitro have been produced and studied by various technics. This work has been done by the student assistant Lemieux. The results are being evaluated and will be published in the near future.

The comparison of these caries in vitro with natural lesions will allow to determine the characteristics of progressive caries and to distinguish between shifting of minerals in the lesion and true remineralisation. This work will be prepared for publication in few months.

The Faculty increased further my teaching program and that slows down the research work. More progress is anticipated during the summer vacation.

Z. Mezl
Z. Mezl
University of Montreal

"AN EVALUATION OF METHODS FOR THE FIXATION
AND STAINING OF GLYCOGEN"

by

J. R. TROTT

Associate Professor of Oral Biology
Faculty of Dentistry
University of Manitoba
Winnipeg

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SUMMARY

Blocks of liver from eight, one-month-old Sprague Dawley rats were fixed in the following fixatives:- 10% Neutral Formalin, 10% Formalin, Formo-Saline, Alcohol Formalin, Acetic Alcohol Formalin, Bouin's, Carnoy's and Rossman's for the following times:- 24 hours, 48 hours, seven days, two weeks, one month and three months. Sections were cut from these specimens and stained by the P.A.S. (Periodic Acid Schiff) reaction, and the following observations were made: Intensity of glycogen, degree of polarization, clarity of the stain and the distribution of glycogen in the sections. It is concluded from these experiments that Acetic Alcohol Formalin fixation probably gives the most reliable and consistent results, whilst Bouin's Fixative is a very close second choice. Rossman's, Carnoy's and Alcohol-Formalin fixatives were preferred to 10% Neutral Formalin, 10% Formalin and Formo-Saline.

Only the P.A.S. stain has been employed so far but it is intended to observe the results with the Best Carmine stain and to compare them with the P.A.S. stain.

The present findings are in closer agreement to Lillie (1947) than to Valence-Owens (1948), though both investigators used the Best Carmine method of staining.

"AN EVALUATION OF METHODS FOR THE FIXATION AND
STAINING OF GLYCOGEN"

INTRODUCTION

The preservation and demonstration of glycogen in tissues for histochemical examination has long been a problem. The problem is two-fold. First the glycogen must be adequately fixed in the tissues so that subsequent processing will remove as little glycogen as possible. Secondly the staining method must show the glycogen clearly and be specific for it.

Pearse (1960) points out that there are a large variety of glycogens occurring naturally of different polymerization and that probably only the higher polymerized glycogens are retained in the tissues. Valence-Owens (1948) demonstrated that sections taken from rabbits livers, fixed in 4% formaldehyde, showed glycogen just as well as those taken from livers fixed in alcohol or picric-alcohol fixatives. Also he found that it made little difference to the amount of glycogen demonstrable in the sections how long the tissues had been retained in fixative for periods from 24 hours to 38 days. Lillie (1947), on the other hand, could find little or no glycogen in sections taken from rat livers fixed in Neutral Formalin or in Acetic Acid-Formalin and stated that he preferred Carnoy's solution for fixation if glycogen was to be preserved. When the tissues were placed in Alcohol fixatives, the sections showed polarization or streaming of the glycogen granules toward one end of the cells. Pearce (1960). It was also noticed that a pronounced difference in the amount of glycogen was seen in the sections taken at different depths of the block. In a previous study, Trott (1957) demonstrated glycogen in the gingival epithelium when 10% Formaldehyde fixation was used, but noted at the time that

there was marked polarization of the glycogen granules, as well as a wide variation in the amount of glycogen demonstrable in the sections.

Because these variations have been observed in the preservation of glycogen, a study was made using various common fixatives, and an evaluation made of their effectiveness. Although a number of freezing methods are available, it appeared desirable to investigate routine methods of glycogen fixation only, as these were the ones which we were most likely to employ in our laboratory.

The problem of staining was studied in a like manner. Although there are a number of satisfactory methods available it would seem that the most commonly used are the P.A.S. (Periodic Acid Schiff) reaction of McManus and Mowry (1960) and Best's Carmine (Pearse 1960). Pearse voices a preference for the Best's Carmine method and although the stain is empirical, he feels it is of value. On the other hand, Trott (1957) found that the P.A.S. reaction gave satisfactory results in his study of the gingivae.

MATERIAL AND METHODS

It is likely that future work will be performed on rats to determine the distribution and content of glycogen in other tissues. Because Whistler and Smart (1953) showed that the liver has the highest concentration of glycogen of all tissues, eight one-month-old healthy Sprague Dawley rats were killed. The livers were removed, quickly sliced into lobes or pieces approximately 10 mm x 5 mm x 5 mm and placed in the appropriate fixatives for periods of 24 hours, 48 hours, 1 week, 2 weeks, 1 month and 3 months. (Lillie (1954), Appendix "A". The rats were not fed any special diet before being sacrificed.

The slices of liver were removed from the fixatives after the above times, and processed as in Appendix "B". Ten sections were cut at 6 - 7 μ ;

five were stained by the P.A.S. reaction of McManus and Mowry (1960), and five by the Best Carmine method.

In each batch of slides being stained was an extra section that had been first treated by a buffered diastase solution for 30 minutes as recommended by McManus and Mowry (1960), Appendix "C". It was found that this was the only effective method of removing glycogen from the sections other than saliva. (Fig.I). A note was kept of how long the diastase solution was effective in removing the glycogen from the sections by comparing a treated slide with untreated ones. It was found that the diastase solution became unreliable after about two hours at a temperature of 37°C. To determine if the Schiff reagent was working satisfactorily, a control slide of gastric mucosa was run through with every batch of slides and it was compared with one that had been treated while the solution was fresh. The Schiff reagent was kept in the refrigerator when not in use, but even so the results could have been unpredictable unless a known slide from the gastric mucosa was stained at the same time as the test slides. The sections of liver were then examined and the following points were noted for each slide.

QUALITY OF SECTION: It is realized that many other factors besides fixation have a bearing on the quality of the sections produced, such as the sharpness of the knife. However, in an experimental group such as this where the dehydration, clearing and wax embedding were all standard for time and temperature, and most of the blocks were cut by one person, it may be possible to say that one type of fixation gives easier material to section, and as a result better slides were produced. Very arbitrarily then the sections were classified as poor, fair or good depending upon the number of tears and folds in the section.

AMOUNT OF GLYCOGEN PRESENT: An overall view of the section was taken and a general idea obtained of the intensity of the glycogen staining. A rough grading of 0, +, ++, +++, ++++ was used as a scoring index - 0 being no glycogen present at all, to ++++, a heavy general distribution of glycogen. It is hoped by this means to see if there was any variation in the amount of glycogen present after varying times in each fixative. Also it was hoped to see if there was any difference from one fixative to another at any one time, or over a period of time. It was also hoped to make some comparison between the P.A.S. and the Best Carmine stain.

DISTRIBUTION OF STAINING: Because previous workers found differences in the staining reaction for glycogen at varying levels in the block, it seemed possible that the centre of a piece of tissue might be less well fixed than the periphery. Similarly a patchy distribution of glycogen might be observed in various parts of the section due to uneven infiltration of the fixative. A note was therefore made of any unusual distribution of the staining in the sections.

POLARIZATION: Streaming of the glycogen granules towards one pole of a cell has been reported on previous occasions hence a note was made on how extreme this was and whether it varied in different areas of the same section.

CLARITY OF STAINING: It is possible that the various fixatives used might influence the clarity of the glycogen as demonstrated by the stains. It was also possible to make a comparison of the two stains that were used. Again, a purely arbitrary basis was used for assessment such as poor, fair or good.

RESULTS

TWENTY-FOUR HOURS: At the end of 24 hours it was difficult to choose which

fixative gave the greatest amount of glycogen in the sections, with the exception of Rossman's, which showed very little glycogen in the sections that were examined (Table I). The remaining fixatives gave sections with an intensity of +++ or more. It is interesting to notice that there was a greater intensity of glycogen staining at the periphery of sections when the specimens had been fixed in 10% Formalin, Formol-Saline and Alcohol Formalin, than with the other fixatives. Acetic Acid Alcohol fixed tissues gave sections with the most even staining throughout, even though the polarization was quite marked, with the exception of sections taken from tissues fixed in 10% Formalin, all the others showed quite marked polarization. The quality of the sections was only fair; 10% Formalin and Neutral 10% Formalin fixed specimens produced the best sections.

FORTY-EIGHT HOURS: At the end of 48 hours some quite marked changes were apparent. Poor sections were noticed from specimens fixed with 10% Formalin, Formol Saline, Rossman's and Carnoy's fixatives, whilst only fair sections were produced from the remaining fixatives. With regard to the intensity of staining, there were even more marked differences (Table I). Carnoy's 10% Formalin and Neutral 10% Formalin fixative tissues gave sections with only ++ glycogen present, whilst sections from tissues fixed in Acetic Alcohol Formalin gave +++ and Bouin's and Rossman's gave +++. When +++ or more glycogen was found in the sections, the only group which did not show any marked polarization were those taken from tissues fixed in Rossman's (Table II). Acetic Alcohol Formalin and Bouin's fixative specimens gave sections which showed more polarization at the periphery than at the centre, whilst those sections taken from Alcohol

Formalin fixed tissues showed a generalized polarization. The clarity of the stain was maintained in all the sections even though the size of the granules varied in sections from different fixatives. Acetic Alcohol Formalin, Bouin's, Rossman's and Alcohol Formalin fixed tissues gave sections with large heavily stained granules, whilst those from tissues fixed in Carnoy's, 10% Formalin, Neutral 10% Formalin and Formol Saline, gave sections which showed small granules, often coalescing but still quite clearly stained.

SEVEN DAYS: The only poor sections produced at this stage were from specimens fixed by Formol Saline, the remaining fixatives gave either fair or good sections. With regard to the intensity of glycogen staining Acetic Alcohol Formalin, Bouin's and Carnoy's fixed specimens gave sections with a ++++ amount of glycogen present (Table I). Specimens from the remaining fixatives; 10% Formalin, Neutral 10% Formalin, and Formol Saline gave sections with only + or ++ glycogen present. Polarization was quite marked in these sections with +++ glycogen or more and it was more pronounced at the periphery than the centre (Table II). However, in sections taken from Alcohol Formalin fixed tissues, it was noticed that polarization was more marked in the centre than at the periphery. Those sections which showed the least amount of glycogen present also showed the least amount of polarization. Glycogen was fairly evenly distributed throughout the sections taken from specimens fixed in Alcohol Formalin, Rossman's, Carnoy's, Bouin's and Acetic Alcohol Formalin, whilst in those taken from specimens fixed in 10% Formalin and Neutral 10% Formalin showed a concentration either at the periphery or in patchy areas throughout the slide (Table IV). It was found that these sections showing +++ or more glycogen had large well stained granules intracellularly, whereas sections taken from specimens fixed in 10% Formalin and Formol Saline showed

small indistinct and often coalescing granules which made it difficult to recognize the glycogen easily and clearly (Table III).

TWO WEEKS: At the end of this period it was found that Bouin's and Rossman's fixativized tissues gave sections with +++ glycogen, and Acetic Alcohol Formalin, Carnoy's and Alcohol Formalin fixed tissues gave sections with ++++ glycogen present (Table I). Tissues fixed in the remaining fixatives gave sections showing only a little glycogen. Rossman's, Neutral 10% Formalin and Formol Saline fixatives gave poor sections, the remainder were fair to good in quality. Polarization was marked in sections taken from Acetic Alcohol Formalin and Rossman's fixed specimens; and in those sections taken from Bouin's and Carnoy's fixed tissues, the polarization was more marked in the periphery than the centre (Table II). The only fixative which gave little or no polarization in the section and still showed quite heavy amounts of glycogen was Alcohol Formalin. Tissues fixativized in Neutral 10% Formalin, Formol Saline and 10% Formalin showed little polarization in the sections. Sections taken from tissues fixed in Rossman's, Formal Saline and 10% Formalin, when stained with the P.A.S. reaction showed small intracellular granules of glycogen which had coalesced to some extent and were not easily distinguishable. Sections taken from specimens fixed in any of the other fixatives showed P.A.S. positive staining granules clearly within the cells (Table III).

ONE MONTH: All the sections in this group were classified as fair or good in quality. Tissues fixed in Acetic Alcohol Formalin gave sections with a glycogen intensity of ++++. Bouin's, Carnoy's and Rossman's fixed specimens gave sections with +++, whilst the remaining fixatives gave sections which showed considerably less glycogen (Table I). Polarization was marked in sections

taken from Acetic Alcohol Formalin and Bouin's fixed specimens, and it was more prominent in the periphery than in the centre of these sections (Table II). Carnoy's fixed specimens gave little polarization in the sections but the general staining was patchy and uneven. Rossman's fixed specimens gave sections with a patchy distribution of glycogen and the polarization was more marked at the periphery than the centre.

The clarity of the staining was more marked in those sections where the granules were larger and not coalescing than in the sections where the granules were smaller, indistinct and coalescing. Granules were larger and more distinct in sections taken from specimens fixed in Acetic Alcohol Formalin, Bouin's, Carnoy's and Rossman's, whereas they were smaller and more indistinct in sections taken from specimens fixed in 10% Formalin, Formol Saline and Neutral Formalin (Table III).

THREE MONTHS: Rossman's fixation was the only one which gave a specimen from which poor sections were obtained, all the others were classified as either fair or good in quality. Carnoy's fixed tissue gave sections which showed +++ amount of glycogen present, whilst ++ amount of glycogen was seen in sections taken from specimens fixed in Alcohol Formalin, Acetic Alcohol Formalin, Bouin's and Rossman's (Table I). Sections taken from specimens fixed in the remaining fixatives gave very poor results and the amount of glycogen observed was only +. Polarization was quite marked in sections taken from specimens fixed in Alcohol Formalin, Acetic Alcohol Formalin, Bouin's, Carnoy's and Rossman's, and it was more pronounced in the periphery than the centre of the section (Table II). On the whole the distribution of the glycogen was generalized in those sections where there was +++ or more glycogen present but patchy in sections taken from

specimens fixed in Neutral 10% Formalin, 10% Formalin and Formol Saline (Table IV). The clarity of the staining was found to be good in all sections even when there was very little glycogen present (Table III). Although the granules were small in some cells they did not appear to have coalesced to the same extent as was noted previously.

DISCUSSION AND CONCLUSIONS

EFFECT OF FIXATION TIME ON THE CUTTING AND QUALITY OF THE SECTIONS: The length of fixation and the type of fixation used seemed to make little difference to the cutting of the sections. Nearly all blocks were difficult to cut satisfactorily and many of them had to have their surfaces soaked in an equal quantity of a glycerine and alcohol solution before adequate sections could be obtained. Generally speaking specimens fixed in the Alcohol group gave better sections than those obtained from the Formalin fixed specimens.

EFFECT OF FIXATION TIME ON THE INTENSITY OF GLYCOGEN STAINING BY THE P.A.S. REACTION:

It will be noticed from Table I, that the simple commonly used Formalin fixatives; Neutral 10% Formalin, 10% Formalin and Formol Saline are adequate up to 24 hours for the fixation of glycogen (Fig.2), but as the time increases the amount of glycogen which is observed in the sections decreases quite markedly at one week and from then until 3 months there is little further deterioration in the amount of glycogen found (Fig.3). Alcohol Formalin, Rossman's and Carnoy's fixatives give reasonably good fixation of glycogen from 24 hours to three months, but the results are inclined to be a little inconsistent, for no apparent reason.

There would seem little to choose between Bouin's and Acetic Alcohol Formalin as fixatives, with regard to the amount of glycogen which is stained by the P.A.S. reaction. Both of them retain sufficient glycogen in the specimen

so that sections show +++ or more consistently from 24 hours fixation to three months. Although it must be agreed that sometimes it is a little difficult to judge whether a section contains +++ or ++++ of glycogen, it would appear from Table I, that perhaps Acetic Alcohol Formalin (Figs 4 & 5), is slightly more efficient than Bouin's as a fixative.

EFFECT OF FIXATION TIME ON POLARIZATION: The results here regarding polarization are similar to those found with regard to the intensity of the glycogen found in the sections (Table II). Tissues fixed in Formalin based fixatives such as Neutral 10% Formalin, 10% Formalin, and Formol Saline all gave the least amount of polarization in the sections when compared with those sections obtained from tissues fixed in Acetic Alcohol Formalin (Fig.6) and Bouin's fixatives which produced a marked polarization. Tissues taken from the other three fixatives, Alcohol Formalin, Rossman's and Carnoy's varied in their effect on polarization found in the sections. On some occasions it would be quite slight and hardly noticeable whilst on other occasions it would be as marked as in sections taken from Acetic Alcohol or Bouin's fixed specimens.

It is interesting to notice that when the polarization is quite noticeable, that it is more marked at the periphery of the sections than at the centre and that this occurs more after 24 hours fixation. The longer the fixation times, the more marked the peripheral polarization, whereas those sections showing a generalized polarization become more of a rarity.

It is difficult from this series to ascertain the cause of the polarization phenomena. All the specimens were treated at the same room temperature, approximately 68°F, so that if the temperature had a direct bearing, it should have effected the Formalin based fixed specimens more than has been observed.

Length of fixation does not appear to have any bearing on the phenomena, as those fixatives which show a marked polarization at the end of 48 hours, also do the same at three months and the reverse also holds true. The direction and speed of penetration of the fixative into the specimen was considered as a possible cause for this phenomena, because a number of sections showed a more marked peripheral than central polarization. This however would not appear to be the case as sections taken at the end of 48 hours from Acetic Alcohol Formalin and Bouin's fixed specimens showed this tendency, and they also showed the same trend at the end of three months. Similarly, the direction of penetration of the fixative would not appear to have much effect. Because if the polarization was related to the manner in which the fixative entered the specimen, then not only should it be quite pronounced at the periphery of the section but also around the large blood vessels which transverse the liver so freely. There was no apparent polarization effect around the vessels. Also there was no constant direction for the polarization. In one area of the section, it might appear to be going towards the centre and then quite suddenly in another area the direction would be reversed. The direction of entry of the fixative cannot be entirely ruled out as a possible cause, as when the periphery of a section showed this phenomena the polarization was usually towards the centre of the section. It was past this peripheral boundary when the direction of polarization appeared so inconsistent.

There seems little doubt that the type of fixative used has a part to play. As can be seen from Table II, there is more marked polarization in the sections taken from tissues with the Alcohol based fixatives than with the Formalin based fixatives.

For routine purposes it would seem more important that one can rely

on the fixative for retaining the glycogen in the tissues rather than the degree of polarization. However, if one wanted to observe more accurately the position of the glycogen in the cells and its relationship to other features of the section, then polarization could be a problem.

EFFECT OF FIXATION TIME ON THE CLARITY OF GLYCOGEN AS DEMONSTRATED BY THE P.A.S.

REACTION: It must be remembered that the P.A.S. reaction will stain other substances besides glycogen, and often the red granules of glycogen are not easy to detect in the cells against a similarly stained background. It also appears that the ease with which glycogen can be observed in the cells depends upon the size of these granules as well as their staining quality. These two factors then are considered together in assessing the clarity with which glycogen can be observed in the sections. It will be noticed from Table III, that up to 48 hours most of the fixatives give reasonable clarity of glycogen staining in sections. Even by this time the Formalin based fixatives are beginning to show a deterioration in clarity which is maintained up to a month and for some reason is not so apparent at three months. This deterioration is largely due to the smallness of the glycogen granules, their propensity to coalesce and the lack of stain intensity.

Alcohol Formalin, Acetic Alcohol Formalin and Bouin's fixed specimens appeared to give consistently good results in the sections, while Carnoy's and Rossman's fixed specimens gave sections where the results were a little unpredictable.

THE EFFECT OF FIXATION TIME ON THE DISTRIBUTION OF GLYCOGEN IN SECTIONS: In considering the distribution of glycogen in the sections, an attempt was made to ignore the intensity of the stain and the polarization and to try and observe whether some sections showed a more consistent distribution of glycogen than others.

From Table IV it would appear that the only fixative which gave consistent generalized glycogen in the sections was Acetic Alcohol Formalin.

Bouin's, Carnoy's, Rossman's and Alcohol Formalin on the whole gave reasonably good results but on one or two occasions either there was more glycogen at the periphery or it appeared to concentrate in patches within the specimen and be uneven in its distribution (Figs. 7 & 8). Neutral 10% Formalin, 10% Formalin and Formol Saline gave even more inconsistent results than any of the above fixatives.

These inconsistencies do not appear to be related to the length of time of fixation. A possibility that might have to be considered is that the livers in the various animals were at different degrees of activity when the animals were sacrificed, so that a more generalized distribution of glycogen may be found in a liver under resting conditions and a patchy distribution under active conditions. This would not appear to be a strong possibility as the liver specimens were placed at random in the fixatives, as the animals were sacrificed, for each time period, rather than all in one particular fixative, and then left for the appropriate time. So that the patchy and peripheral distribution is probably more of a fixation artifact than a functional difference in the glycogen content of the livers.

This is as far as the work has been taken at present. A summary is given in the earlier part of the report and it is intended to proceed along the lines indicated in the application submitted.

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APPENDIX "A"FIXATIVES

10% Formalin

Concentrated formaldehyde Solution 37-40%)	100 cc
Tap Water	900 cc

Formol Saline

37-40% Formaldehyde Solution	100 cc
Sodium Chloride	8.5 gm
Tap Water	900 cc

Alcoholic Formalin

37-40% Formaldehyde Solution	100 cc
95% Alcohol	900 cc

Acetic Alcohol Formalin

37-40% Formaldehyde Solution	10 cc
Glacial Acetic Acid	5 cc
100% Alcohol	85 cc

Rossman Fluid

90 vols of 100% Alcohol saturated with Picric Acid and 10 vols of neutralized commercial formalin

Carnoy's Fluid

100% Alcohol	60 cc
Chloroform	30 cc
Glacial Acetic Acid	10 cc

Bouin's Fluid

1.2% saturated Picric Acid solution (Aqueous)	75 cc
40% Formalin	25 cc
Glacial Acetic Acid	5 cc

Neutral 10% Formalin

37-40% Formaldehyde Solution	100 cc
Tap Water	900 cc
Calcium Carbonate to excess	

APPENDIX "B"PROCESSING OF TISSUES

Following fixation for the appropriate time interval the specimens were then passed through an automatic processor which was set as follows:

1 hour	50% Alcohol
1 hour	70% Alcohol
1 hour	80% Alcohol
1 hour	95% Alcohol
1 hour	95% Alcohol
1 hour	Absolute Alcohol
1 hour	Absolute Alcohol
1½ hours	Benzene Alcohol
1½ hours	Benzene Alcohol
1½ hours	Wax at 54°C
1 hour	Wax at 54°C

The specimens were then embedded in Tissuemat at 54-56°C

Following cutting, the wax ribbons were placed on a water bath with a temperature of 48°C and then mounted on a slide smeared with albumin before being dried in an oven at about 45°C for two or more hours. The slides were then passed through two changes of Xylol for 2 to 5 minutes, then into two changes of 95% Alcohol for 2 to 3 minutes before being placed in running water. The slide was then either treated with the Malt Diastase Buffer or the F.A.S. reaction.

APPENDIX "C"MALT DIASTASE METHOD

1. Diastase-Buffer Solvent

Freshly boiled distilled water	1000 ml
Anhydrous disodium phosphate Na_2HPO_4	0.28 Gm
Monosodium phosphate ($\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$)	1.97 Gm
Sodium Chloride	8.00 Gm
Dissolve and add crystals of thymol to prevent mold. The buffer is stable for months.	

2. Malt Diastase Solution

Diastase Buffer-Solvent	100 ml
Malt Diastase, U.S.P. IX (Merck's)	0.1 Gm

Prepare just before use and filter to remove starch granules.

Warm to 37°C before introducing slides. While a 1 per cent diastase solution was used originally, 0.1 per cent is adequate and reduces side action by contaminative enzymes, presumably proteases.

TABLE I

COMPARISON OF INTENSITY OF GLYCOGEN STAINING

	'24 hours	'48 hours	'7 days	'2 weeks	'1 month	'3 months
Neutral 10%	+++	++	+	+	++	+
10% Formalin	+++	++	++	+	+	+
Formol Saline	+++	++	+	+	+	+
Alcohol Formalin	+++	+++	+++	+++	++	+++
Acetic Alcohol Formalin	+++	+++	+++	+++	+++	+++
Bouin's	+++	+++	+++	++	+++	+++
Carnoy's	+++	++	+++	++++	+++	++++
Rossman's	+	++++	+++	+++	+++	+++

This Table shows the amount of Glycogen found in sections taken from rats livers, stained by the P.A.S. reaction, following fixation in various fixatives from 24 hours to 3 months.

TABLE II

POLARIZATION

	24 hours	48 hours	7 days	2 weeks	1 month	3 months
Neutral 10%	Marked	Slight	Slight	Slight	Slight	Slight
10% Formalin	Nil	Slight	Slight	Slight	Slight	Slight
Formol Saline	Marked in Patches	Slight	Slight	Slight	Slight	Marked at Periphery
Alcohol Formalin	Marked in Centre	Marked	Marked at Periphery	Slight	Marked	Marked at Periphery
Acetic Alcohol Formalin	Marked	Marked at Periphery	Marked at Periphery	Marked	Marked at Periphery	Marked at Periphery
Bouin's	Marked	Marked at Periphery	Marked at Periphery	Marked at Periphery	Marked at Periphery	Marked at Periphery
Carnoy's	Marked	Marked at Periphery	Marked at Periphery	Marked at Periphery	Slight	Marked at Periphery
Rossmann's	Slight	Slight	Marked at Periphery	Slight	Marked at Periphery	Marked at Periphery

This Table shows the degree of polarization found in sections taken from rats livers fixed in various fixatives from 24 hours to 3 months.

TABLE III
CLARITY OF GLYCOGEN

Fixatives	24 hours	48 hours	7 days	2 weeks	1 month	3 months
Neutral 10% Formalin	fair	fair	poor	poor	fair	good
10% Formalin	good	fair	poor	poor	poor	good
Formol Saline	good	fair	poor	poor	poor	fair
Alcohol Formalin	good	good	good	good	good	good
Acetic Alcohol Formalin	good	good	good	good	good	good
Bouin's	good	good	good	good	good	good
Carnoy's	good	poor	fair	good	good	good
Rossman's	poor	fair	good	fair	good	fair

This Table shows the clarity with which glycogen was observed in sections when stained by the P. A. S. reaction, in rat livers fixed in various fixatives from 24 hours to 3 months.

TABLE II
LYSER II

TABLE IV
DISTRIBUTION OF GLYCOGEN

Fixatives	24 hours	48 hours	7 days	2 weeks	1 month	3 months
Neutral 10% Formalin	More at Periphery	More at Periphery	More at Periphery	More at Periphery	Patchy	Patchy
10% Formalin	More at Periphery	Patchy	More at Periphery	Generalized	Patchy	Patchy
Formol Saline	More at Periphery	Generalized	Generalized	Patchy	Generalized	More at Periphery
Alcohol Formalin	More at Periphery	More at Periphery	Generalized	Generalized	More at Periphery	Generalized
Acetic Alcohol Formalin	Generalized	Generalized	Generalized	Generalized	Generalized	Generalized
Bouin's	Patchy	Generalized	Generalized	More at Periphery	Generalized	Generalized
Carnoy's	Patchy	Generalized	Generalized	Generalized	Patchy	Generalized
Rossman's	Generalized	Generalized	Generalized	Generalized	Patchy	Generalized

This Table shows the distribution of Glycogen found in sections taken from rats livers fixed in various fixatives over periods of time from 24 hours to 3 months.

LEGENDS TO ILLUSTRATIONS

- FIG. 1. A section of liver showing no glycogen present following the use of diastase. Fixation in Acetic Alcohol Formalin for three months. Stain P.A.S. Mag x 200.
- FIG. 2. A section of liver showing the amount of glycogen present following 24 hours fixation in 10% Formalin. Stain P.A.S. Mag x 200.
- FIG. 3. A section of liver showing the decreased amount of glycogen present following three months fixation in 10% Formalin. Stain P.A.S. Mag x 200.
- FIG. 4. A section of liver showing the amount of glycogen present following 24 hours fixation in Acetic Alcohol Formalin. Stain P.A.S. Mag 200.
- FIG. 5. A section of liver showing the amount of glycogen present following three months fixation in Acetic Alcohol Formalin. Stain P.A.S. Mag x 200.
- FIG. 6. A section of liver showing the degree of polarization that occurs at the periphery of a section. Fixation in Acetic Alcohol Formalin. Stain P:A.S. Mag x 200.
- FIG. 7. A section of liver showing the uneven distribution of glycogen. Fixation in Carnoy's solution for 1 month. Stain P.A.S. Mag x 200.
- FIG. 8. A section of liver showing the concentration of glycogen at the periphery. Fixation 10% Formalin for 24 hours. Stain P.A.S. Mag x 200.

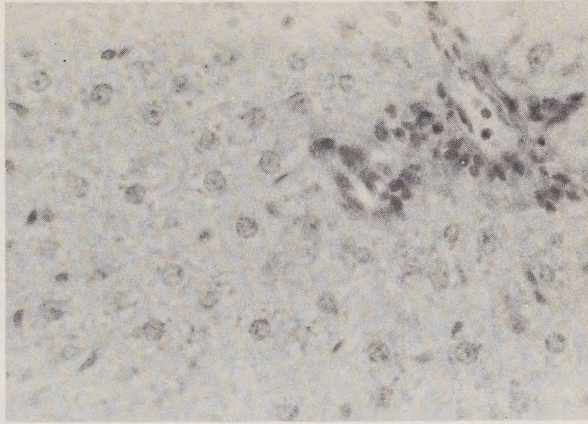


FIGURE 1.

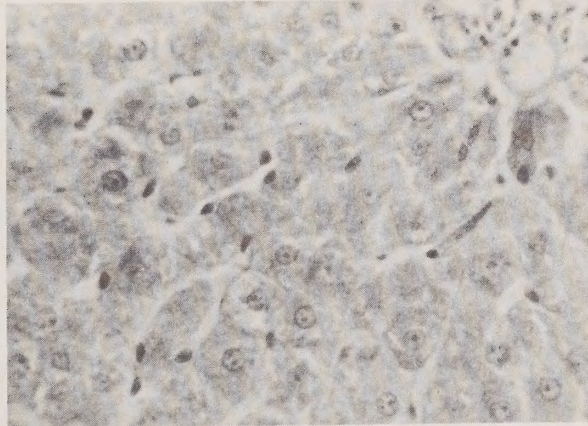


FIGURE 2.

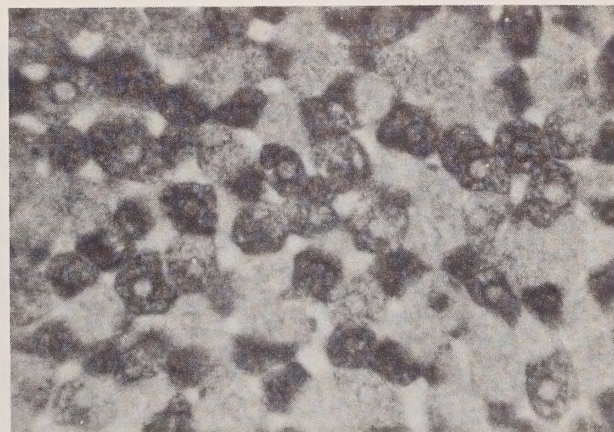


FIGURE 3.

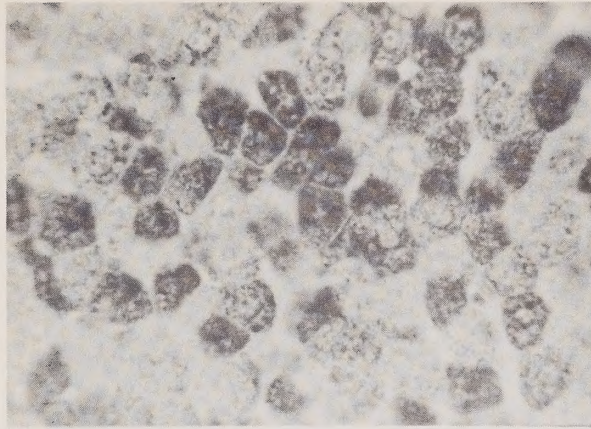


FIGURE 4.

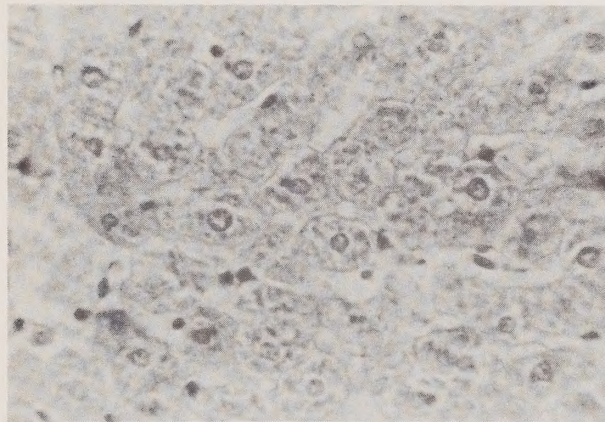


FIGURE 5.

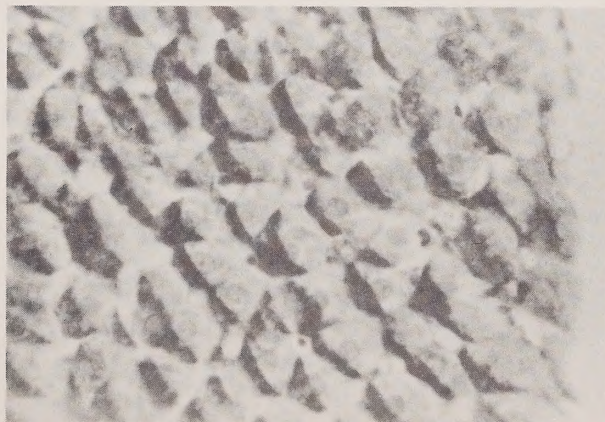


FIGURE 6.

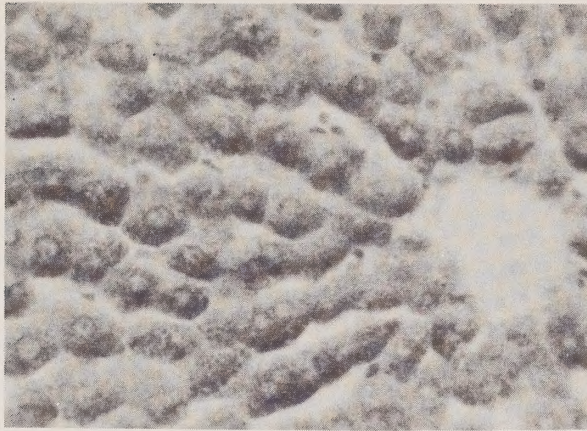


FIGURE 7.

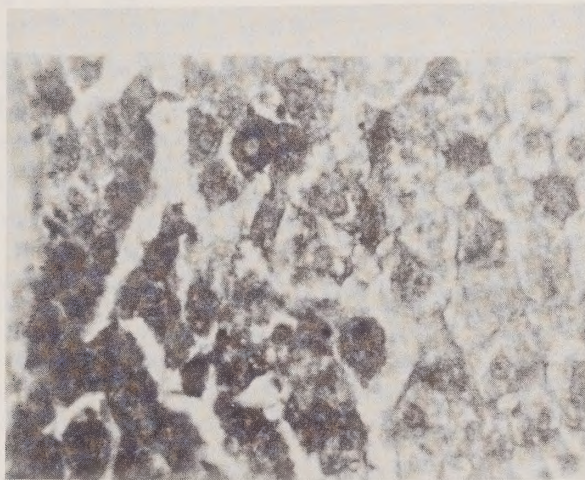


FIGURE 8.

August 23, 1960

REPORT TOTHE ASSOCIATE COMMITTEE ON DENTAL RESEARCHNATIONAL RESEARCH COUNCIL OF CANADA

SUBJECT: The study of traumatic (surgical) tooth movement.

AUTHOR: R. D. Haryett

LABORATORY: Department of Physiology and Pharmacology, University of Alberta.

GRANT NO. D. A. 69

PURPOSE: The experiment was to be carried out in three phases (A, B, and C). The experimental animals were to be used specifically for A and B. However, it was hoped to do an exploratory study (c) on the same animals, which would not have prejudiced the findings in A or B.

Phase A. To study the tendencies of relapse, if any, in traumatically rotated incisors of adult rhesus monkeys. This phase has been carried out by Dr. Haryett.

Phase B. To study the effects of traumatic tooth rotations on the pulp vitality and ultimate integrity of the periodontal membrane.

Dr. McMurchy is presently completing the histologic study.

Phase C. To study the basic oral reflexes in decerebrated adult rhesus monkeys by electromyography. It was planned to explore the physiology of oral reflexes when the animals were being sacrificed. Unfortunately, it was not possible to carry out this phase of the study as suitable electromyographic equipment was not available.

The grant includes a histologic study by Dr. K. A. McMurchy to be carried out when the animals are sacrificed. This aspect of the study is presently under way.

MATERIAL AND METHOD:

Experimental Procedures: (see report of January 1958 on Grant No. D. A. 69)

Four adult rhesus monkeys (weight approximately eight and one half pounds) were used in this study. Each animal was anaesthetized by intra peritoneal injections of nembutal (Fig. 1) A level of 25-30 mg. of nembutal per kilogram of body weight gave adequate relaxation for an operating period of three hours. One three hundredth grain of atropine was added to and injected with the nembutal to reduce salivation. As soon as anaesthesia was obtained, the monkey was placed in a specially designed dental chair (Figs. 2, 3, 4) for operative procedures and intubated orally. (Fig. 5)

The operative procedures were carried out on each experimental animal in the following steps:

- Step one:
- a. Maxillary and mandibular impressions for study models.
 - b. Roentgenograms of maxillary incisors. The x-ray technique was standardized as best as possible without a head holder. All occlusal films were taken at an angulation of 65° to the maxillary occlusal plane, with a setting of 65KVP, 10 ma, and exposure duration of 2 seconds. (Fig. 6)
 - c. Brass wire separation between the teeth to be banded. (Fig. 7)
 - d. Photographs
- Step two:
- a. Separation wires removed
 - b. Bands fitted on the maxillary incisors, canines, first bicuspid, and first molars
 - c. Separation replaced

- Step three:
- a. Separation wires removed
 - b. Cementation of bands on canines, first bicusps, first molars, and incisors to be used as controls (left lateral and right central incisors).
 - c. Maxillary right lateral incisor traumatically rotated mesio-labially until the periodontal membrane was broken (approximately 20° - 30°).
 - d. Maxillary left central incisor traumatically rotated mesio-labially until the periodontal membrane was broken (approximately 20° - 30°).
 - e. Bands cemented on rotated incisors.
 - f. Passive .020 round stainless steel labial arch ligated with .012 ligature wire to stabilize the rotated teeth until healing of the periodontal membrane occurs. (Figs. 8,9)
 - g. Photographs and roentgenograms

- Step four:
- a. Monkeys were checked at intervals for loose bands and ligatures and progress roentgenograms of the maxillary incisors.
 - b. After four months had elapsed, all bands were removed. Progress roentgenograms, photographs (Fig. 10) and impressions for study models were taken.

- Step five:
- a. Progress records taken approximately six months after the bands were removed.

- Step six:
- a. Final progress records were taken just before the animals were sacrificed.

- Step seven:
- a. Step seven is the histologic aspect of the study to be carried out by Dr. McMurchy when the animals were sacrificed.

Table I summarizes the important steps of the experiment and gives the particulars of the teeth which were rotated, the exact duration in months between each step, and the numbering of the study models.

Before presenting the findings, I would like to make a few brief observations about the experimental procedure.

1. It is very difficult to rotate an incisor tooth and at the same time maintain its correct elevation in the dental socket. If the root form was a perfect cone fitting in a cone-type of dental socket, the rotation would be simple. However, since the root form is not an ideal cone and is usually slightly curved, there seems to be a tendency when rotating an incisor for the tooth to emerge from its socket. The same effect would be experienced if one was twisting a corkscrew backwards in a piece of wood and at the same time attempting to prevent the corkscrew from emerging from its hole.

In two attempts at rotation (see table 1 - monkeys #1 and #3,) an incisor was inadvertently extracted as the crown popped through the beaks of the forcep. Further incidences of this nature were prevented by fitting a small piece of a rubber cork within the confines of the beaks. (Fig. 11) On another instance of attempted rotation, the crown was fractured from the root (table 1 - monkey #3).

2. As a tooth is rotated, the axial inclination of the tooth also changes due to the irregular root form. When an attempt is made to align a rotated tooth in the dental arch by rotation alone, the alteration in axial inclination would create a labial, lingual, mesial, or distal angulation (Fig. 12.).

RESULTS:

A. Roentgenographic findings:

Occlusal roentgenograms were taken at each major step of the experimental procedures (Table I). Pictures of the roentgenograms for each monkey are presented in Figures 13, 14, 15, and 16. The diagnostic interpretation by the author was based on the integrity of the root surface, periodontal membrane and lamina dura (Tables II, III, IV and V). A summary of the radiographic findings is presented in Table VI.

Analysis - The Chi-square Goodness of Fit* has been used to reduce the data in Table VI in a way to permit comparison between the experimental (rotated) maxillary incisors and control (non-rotated) maxillary incisors. The Chi-square value (X^2) is a statistical computation stating whether or not the differences shown are dependent upon real associative factors or whether they have been ascribed to chance. The sum of the Chi-square values indicates the statistical significance and P is a statement of the chances out of 100 of this Chi-square sum being significant. Table VII gives the comparison between pulp pathology in the rotated and control teeth. The probability of pulp pathology of the traumatically rotated incisors of adult rhesus monkeys is significant at the 1% level. Table VIII shows the comparison between apical root resorption in the rotated and control teeth. The probability of apical root resorption of the traumatically rotated incisors of adult rhesus monkeys is significant at the 5% level.

The remaining radiographic observations such as resorption of root surface, internal resorption, and involvement of the floor of nasal cavity were found to be non-significant.

B. Findings on Tooth Rotations as obtained from the study Models:

Impressions were made and study models were prepared at each major step of the experimental procedures (Table I). Occlusal photographs were taken of the maxillary dental casts. (Fig. 17, 18, 19 and 20). Each dental cast was orientated on a moveable support so the tip of the mesio-buccal cusp of the right and left first molars and the incisal edge of the control central incisor were on a horizontal plane. The lens to subject distance was identical for all the photographs taken of the dental casts for each monkey (Fig. 21). Cross hairs on the focusing ground glass were superimposed on the mid-sagittal plane and the tips of the cusps of the right and left canines (Fig. 22). Therefore, the centre of the picture was orientated to the same anatomical landmarks on every dental cast. The proximity of the centre of the picture to the incisors would also reduce the amount of parallax when measuring the angulation of the incisors to the sagittal plane. Consequently the pictures could be developed to the same scale and would be comparable for angular measurements on tooth rotations.

The following method was used to measure the angulation of the maxillary incisors to the sagittal plane. Six enlargements were printed from the photographs of all the dental casts. The angulation of the incisors

were measured directly on the enlargement in the following manner (Fig. 22). The mid-sagittal plane of the palate was determined by drawing a straight line on each photograph through three identical points located on the median raphe. A straight line was then drawn through the most mesial and most distal incisal line angles and projected sufficiently so that a protractor could be used to measure the angulation of the tooth to the mid-sagittal plane. The inside (acute) angle was the angle which was recorded. Six measurements were made for each dental cast and averaged to reduce the error in measurement (Tables IX, X, XI, XII). Table XIII summarizes the mean angular measurements of the maxillary incisors to the mid-sagittal plane. The figures for the remaining statistical analyses are taken from this table.

Analyses:

It is obvious when one studies Table XIII that the angulation of the rotated incisors was altered considerably by the traumatic rotation. Table XIV demonstrates that the rotated teeth relapsed significantly ($P = < 0.05$) through several degrees toward the original angulation after the bands were removed.

It was very unexpected to find that the control teeth were orthodontically rotated ($P = < 0.05$) by the passive stabilizing .020 stainless steel arch (Table XV). After the bands were removed from the control teeth, it was not surprising to find a significant relapse ($P = < 0.01$)

It was not possible to show any significant difference in the amount of relapse of the rotated and control teeth.

One cannot overlook the fact that three of the rotated teeth exfoliated prematurely from root resorption and one (Monkey #3 - right lateral) had the crown fractured from the root during rotation. It would be very difficult to assess how much the loss of these teeth played in the relapse of the remaining incisors. Undoubtedly, it would have some effect.

Another factor which should be recorded but which would probably have very slight effect, if any, on either the roentgenographic findings or angular measurements. Monkey #4 died from unknown causes before it could be sacrificed. However, the animal was placed in refrigeration within six hours and final records were obtained within twenty - four hours. The premature death of Monkey #4 will undoubtedly affect the histologic findings on the pulp.

Summary:

A technique has been developed for carrying out oral experimental procedures on the monkey. The maxillary right lateral and left central incisors were traumatically rotated in four adult female rhesus monkeys.

The maxillary right central and left lateral incisors in the same animal were used as control.

The findings are summarized as follows:

Significant at 95% level

1. Relapse of traumatically rotated teeth.
2. Pulp pathology in traumatically rotated teeth.

3. Apical Root resorption in the traumatically rotated teeth.
4. Orthodontic rotatory movement of controls.
5. Relapse of controls.

Non-Significant :

1. Difference in amount of relapse of traumatically rotated teeth and controls.
2. Difference in the amount of root surface resorption in rotated and controls.
3. Difference in the internal root resorption in rotated and controls.

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TABLE 1

TEETH ROTATED AND TIME INTERVAL FOR RECORDS (STUDY MODELS AND OCCLUSAL ROENTGENOGRAMS)

EXPERIMENTAL ANIMAL Teeth Rotated: Maxilla Only	ORIGINAL RECORDS	TRAUMATIC TOOTH ROTATION	BANDS REMOVED	PROGRESS RECORDS	PROGRESS RECORDS PRIOR TO SACRIFICE	
<u>MONKEY NO. 1.</u>						
Left central extract- ed washed in 70% alcohol replaced in socket and ligated to arch.	DATE	Dec. 1957	Jan. 24, /58	May 27, /58	Feb. 19, /59	October 16, 1959
Right Lateral rotated	Study Model	1A	1B	1C	1D	
	Months Duration		0	4	13	21
<u>MONKEY NO. 2.</u>						
Left Central Rotated	DATE	Jan. 17, /58	Feb. 14, /58	July 15, /58	Feb. 19, /59	October 18, 1959
Right Lateral Rotated	Study Model	2A	2B	2C	2D	
	Months Duration		0	5	12	20
<u>MONKEY NO. 3.</u>						
Left central extract- ed - washed in 70% alcohol - replaced in socket and ligated.	DATE	Apr. 11, /58	April 20, /58	Sept. 25, /58	March 6, /59	October 18, 1959
Right Lateral - crown fractured at neck	Study Model	3A	3B	3C	3D	
	Months Duration		0	5	10 1/2	18
<u>MONKEY NO. 4.</u>						
Left central rotated	DATE	Mar. 21, /58	April 4, /58	Oct. 10, /58	None Taken	September 30, 1958
Right Lateral rotated	Study Model	4A	4B	4D		
	Months Duration		0	6		17 1/2

TABLE II

Teeth: Rotated and Control Maxillary Incisors		Just Prior to Rotation	Roentgenographic Findings		Monkey # One	Just Prior to Sacrifice	Diagnosis
			Bands Removed. Teeth have been rotated and periodontal membrane healed.	Progress Findings			
Left Central: Extracted, washed in 70% alcohol, re-placed in socket and ligated to a passive arch in rotated position	Normal Picture	R.S.: Smooth	R.S.: Approximately one-half of root resorbed	Crown missing, root fragment in socket	Crown missing, root fragmentation in socket	Root resorption and ex-foliation of the tooth crown	
	** R.S.: Smooth	P.M.: Narrow, clearly defined	P.M.: Non-existent				
	*** L.D.: Continuous and clearly defined						
Right Central: Control tooth Ligated to passive arch	Normal Picture	R.S.: Smooth	R.S.: Appears uneven in localized areas of root	R.S.: Uneven in localized areas		Localized resorption along root surface. Unusual thickness of periodontal membrane at apex suggests the possibility of pulp pathology. Abnormal thickness of periodontal membrane on distal aspect of root is probably due to mesial tipping into space where left central incisor was last	
	P.M.: Narrow, clearly defined	L.D.: Continuous and clearly defined	P.M.: Clearly defined, wider on distal aspect of root	P.M.: Clearly defined, wider on distal aspect of root			
	L.D.: Continuous and clearly defined		L.D.: Less clearly defined on distal aspect of root	L.D.: Continuous and clearly defined except at root apex			
Right Lateral: Rotated Ligated to passive arch	Normal Picture	R.S.: Very uneven	R.S.: Very uneven	R.S.: Very uneven		Lack of periodontal membrane and the Lamina dura and the localized area of increased radio-lucency at root apex suggests pulp pathology. Considerable localized surface root resorption	
	R.S.: Smooth	P.M.: Poorly defined, almost non-existent	P.M.: Poorly defined, non-existent at apex of root	P.M.: Poorly defined, non-existent at root apex			
	P.M.: Narrow, clearly defined	L.D.: Continuous, and clearly defined	L.D.: Poorly defined, non-existent at root apex	L.D.: Localized area of increased radio-lucency at root apex			
Left Lateral: Control tooth Ligated to passive arch	Normal Picture	R.S.: Uneven along mesial surface	R.S.: Uneven along mesial surface	R.S.: Uneven along mesial surface of root		Localized surface resorption of root visible otherwise roentgenogram appears normal	
	R.S.: Smooth	P.M.: Poorly defined along mesial aspect of root	P.M.: Clearly defined	P.M.: Clearly defined			
	P.M.: Narrow, clearly defined	L.D.: Poorly defined along mesial aspect of root	L.D.: Clearly defined	L.D.: Clearly defined - appears to be some root resorption at apex			
Time Interval (Months)		0	4	13	21		
Figure							
Study Model (Legend)		1A	1B	1C	1D		

* R.S.: Means root surface

** P.M.: Means periodontal membrane

*** L.D.: Means Lamina dura

TABLE III

Teeth: Rotated and Control Maxillary Incisors

Roentgenographic Findings		Monkey # Two		Diagnosis
Bands Removed.	Teeth have been rotated and periodontal membrane healed	Progress Findings	Just Prior to Sacrifice	
Left Central: Rotated Ligated to passive arch	Normal Picture * R.S.: Smooth ** P.M.: Narrow, clearly defined *** L.D.: Continuous, clearly defined	R.S.: Appears smooth, uneven at apex P.M.: Clearly defined, except at root apex L.D.: Broken at root apex	R.S.: Appears uneven at apex P.M.: Well defined area of increased radio-lucency at apex of root L.D.: Break in continuity of root apex	Root resorption at apex of root. Area of radio-lucency and break in lamina dura suggest pulp pathology
Right Central: Control Ligated to passive arch	Normal Picture R.S.: Smooth P.M.: Narrow, clearly defined L.D.: Continuous, clearly defined	R.S.: Smooth P.M.: Clearly defined, slightly wider at apex L.D.: Continuous, clearly defined	R.S.: P.M. and L.D.: Appear normal in structure	Mild resorption at apex of root. Changes in P.M. and L.D. at apex may be caused by abnormal tooth loading or proximity to pathology at apex of adjacent lateral incisor
Right Lateral: Rotated Ligated to passive arch	Normal Picture R.S.: Smooth P.M.: Narrow, clearly defined L.D.: Continuous, clearly defined	R.S.: Smooth P.M.: Clearly defined, except at apex L.D.: Poorly defined at apex	R.S.: Uneven at apex P.M.: Poorly defined at apex - localized area of increased radio-lucency L.D.: Definite break in continuity at apex	Root resorption at apex of root. Area of radio-lucency and break in lamina dura suggest pulp pathology. Also evidence of internal resorption in apical third.
Left Lateral: Control Ligated to passive arch	Normal Picture R.S.: Smooth P.M.: Narrow, clearly defined L.D.: Continuous, clearly defined	R.S., P.M., and L.D.: Appear normal Apex normal not clearly visible since superimposed by crown of cuspid	R.S., P.M., and L.D.: Poorly defined at apical third of root	Apical third of R.S., P.M. and L.D.: Poorly defined. Appears normal
Time Interval (Months)	0	5	12	20
Figure				
Study Model (Legend)	2A	2B	2C	2D

* R.S.: Means root surface ** P.M.: Means periodontal membrane *** L.D.: Means Lamina dura

TABLE IV

Teeth: Rotated and Control Maxillary Incisors	Roentgenographic Findings		Monkey # Three		Diagnosis
	Just Before Rotation	Teeth Bands Removed.	Progress Findings	Just Prior to Sacrifice	
Left Central: Extracted, washed in 70% alcohol, replaced in socket and ligated to a passive arch in rotated position	Normal Picture	R.S.: Approximately one- half of root resorbed	Tooth Missing	Tooth missing	Root resorption and exfoliation of tooth
	* R.S.: Smooth	P.M.: Non-existent			
	** P.M.: Narrow, clearly defined	L.D.: Non-existent			
	*** L.D.: Continuous, clearly defined				
Right Central: Control Ligated to passive arch	Normal Picture	R.S.: Uneven on apical third of root	R.S. Uneven on apical third of root	R.S.: Uneven on apical third of root	Localized surface resorption of root. Surface on apical third.
	R.S.: Smooth	P.M.: Clearly defined, slightly wider on distal aspect of root and at apex	P.M. Clearly defined,	P.M. & L.D.: appear normal	Increased thickness of perio- dental membrane along distal aspect of the root probably due to mesial tipping of the control incisor into the space formerly occupied by the left central.
	L.D.: Continuous, clearly defined	L.D.: Continuous, clearly defined	slightly wider on distal as- pect of root and at apex		
			L.D. Con- tinuous, clear- ly defined		
Right Lateral: Fractured Crown was fractured at neck during attempted rotation	Normal Picture	R.S., P.M., and L.D.	R.S., P.M., and L.D.: Very poorly defined	R.S., P.M., and L.D.: Very poorly defined	R.S., P.M., and L.D. are poorly defined since root is no longer in function
	R.S.: Smooth	Very poorly defined			
	P.M.: Narrow, clearly defined				
	L.D.: Continuous, clearly defined				
Left Lateral: Control Ligated to passive arch	Normal Picture	R.S., P.M., and L.D.: Poorly defined in apical half of mesial aspect of root	P.M.: ap- pears wider on distal as- pect of root	R.S., P.M., and L.D.: Poorly defined - appear normal	Increased thickness of perio- dental membrane on distal aspect of root probably due to mesial tipping into the space formerly occupied by the left central.
	R.S.: Smooth				
	P.M.: Narrow, clearly defined				
	L.D.: Continuous, clearly defined				
Time Interval (Months)	0	5	10 1/2	18	
Figure					
Study Model (Legend)	3A	3B	3C	3D	

* R.S.: Means root surface.

** P.M.: Means periodontal membrane

*** L.D.: Means Lamina dura

TABLE V

TABLE V						
Teeth: Rotated and Control Maxillary Incisors		Just Before Rotation	Roentgenographic findings		Monkey # Four	Diagnosis
			Bands Removed. Teeth have been rotated and periodon- tal membrane healed.	Progress Findings	Just Prior to Sacrifice	
Left Central: Rotated Ligated to passive arch	Normal Picture		R.S.: Very uneven along distal aspect of root and at root apex.		R.S. Very uneven along distal aspect of root and at root apex.	Localized surface resorption of root resorption on distal aspect of root and at root apex.
	* R.S.: Smooth					
	** P.M.: Narrow, clearly defined		P.M.: Poorly defined along distal aspect of root and at root apex. Localized area of increased radio-lucency at root apex.	Not Available	P.M. Poorly defined along portions of distal aspect of root and at root apex. Localized area of increased radio-lucency at root apex.	Localized area of radio-lucency at root apex suggests pulp pathology. Possible pathologic involvement of floor of nasal cavity.
	*** L.D.: Continuous, clearly defined		L.D.: Definite break in con- tinuity at root apex.		L.D. Definite break in continuity at root apex. Possibility of a break in the continuity of floor of nasal cavity.	
Right Central: Control Ligated to passive arch	Normal Picture		R.S.: Appears uneven in apical one third of root		R.S.: Appears uneven in apical one third of root	Mild surface resorption on apical one third of root, increased
	R.S.: Smooth					thickness of P.M. on mesial
	P.M.: Narrow, clearly defined		P.M.: Slightly wider around apical one third of root	Not	P.M.: Wider on mesial aspect of the apical one third of root	aspect of root apex and poorly
	L.D.: Continuous, clearly defined		L.D.: Appears to have a break in continuity at root apex.	Available	L.D.: Appears to have a break in the continuity of root apex	defined lamina dura probably the result of abnormal tooth leading
Right Lateral: Rotated Ligated to passive arch	Normal Picture		Crown was loose and fell out when the maxillary arch was removed Root tip (approx. 1/3 of root) is vis- ible in socket. R.S., P.M. and L.D. are poorly defined. Small localized area of in- creased radio-lucency vis- ible at root tip - also root resorption visible at root apex.		R.S., P.M., and L.D.: around root tip is well defined. Area of increased radio-lucency is visible at apex of root. Apex of root appears uneven.	Excessive surface resorption on root and internal resorption about middle one third has caused a pathologic fracture of root in this region. Hence crown exfoliated as soon as arch was removed.
	R.S.: Smooth					
	P.M.: Narrow, clearly defined			Not		
	L.D.: Continuous, clearly defined			Available		
Left Lateral: Control Ligated to passive arch	Normal Picture		R.S.: Smooth		R.S., P.M., and L.D.: Are poorly defined at apical third - appear normal	Normal
	R.S.: Smooth					
	P.M.: Narrow, clearly defined		P.M.: Appea-s wider along mesial aspect of root	Not		
	L.D.: Continuous, clearly defined		L.D.: Appears normal al- though less clearly defined.	Available		
Time Interval (Months)		0	6	--	17 1/2	
Figure				--		
Study Model (Legend)		4A	4B	--	4D	

* R.S.: Means root Surface ** P.M.: Means periodontal membrane *** L.D.: Means Lamina dura

TABLE VI

SUMMARY OF RADIOGRAPHIC FINDINGS OF EXPERIMENTAL AND CONTROL TEETH
FROM TABLES 2, 3, 4, 5

EXPERIMENTAL TEETH (7) *					
Rotated Teeth (5)	Pulp Pathology	Apical Root Resorption	Resorption of Root Surface	Internal Resorption	Floor of Nasal Cavity Involved
5	5	5	5	2	1
Extracted Teeth (2) (Replaced in Socket)	2	2 (Root Resor- bed and Crown Exfoliated)	2		
CONTROL TEETH (8)					
Control Teeth (8)	0	1	4	0	0

* 5 Teeth were rotated and ligated to passive wire labial arch

2 Teeth were extracted accidentally, washed in 70% alcohol, replaced in socket and ligated

1 Tooth was fractured at neck; hence couldn't be used

TABLE VII

PULP PATHOLOGY (Radiographic Findings)

	Pulp Pathology	No. Pulp Path.	Total
Rotated Teeth	5	0	5
Non-Rotated (Central) Teeth	0	8	8
TOTAL	5	8	13

Sum of $\chi^2 = 9.12$ $P = < 0.01$

TABLE VIII

APICAL ROOT RESORPTION (Radiographic Findings)

	Apical Root Resorption	No Apical Root Resorption	Total
Rotated Teeth	5	0	5
Non-Rotated (Central) Teeth	1	7	8
TOTAL	6	7	13

Sum of $\chi^2 = 5.85$ $P = < .05$

TABLE IX

ANGULATION OF MAXILLARY INCISORS TO MID-SAGITTAL PLANE

MONKEY #1

	Right Lateral Incisor (Rotated)	Right Central Incisor (Control)	Left Central Incisor (Rotated)	Left Lateral Incisor (Control)
Model 1A	35.0 35.0 38.0 36.5 37.0 35.5	65.50 65.50 76.5 64.5 65.0 65.0	69.0 69.5 69.0 69.5 70.5 69.0	35.0 35.50 36.0 39.0 36.0 36.5
Mean	36.2	65.5	69.4	36.5
Left central extracted -washed 70% alcohol -replaced in socket				
Model 1B	23.0 21.5 23.0 22.5 23.5 22.5	69.0 69.5 69.0 69.5 68.5 69.5	49.0 48.5 46.0 51.0 49.0 49.0	36.0 35.0 36.0 37.0 37.5 36.0
Mean	22.7	69.2	48.8	36.3
Model 1C	31.0 30.5 31.0 30.0 31.0 30.5	73.0 72.0 74.0 74.0 74.0 72.0	Tooth Exfoliated - - - - -	39.0 40.5 40.5 41.0 37.5 39.5
Mean	30.7	73.2		39.7
Model 1D	37.0 36.0 36.0 35.0 37.0 37.0	78.0 77.5 78.5 77.5 77.5 78.0	- - - - - -	39.0 41.0 42.0 41.0 42.5 40.0
Mean	36.3	77.8		40.9

TABLE X

ANGULATION OF MAXILLARY INCISORS TO MID-SAGITTAL PLANE

MONKEY #2

	Right Lateral Incisor (Rotated)	Right Central Incisor (Control)	Left Central Incisor (Rotated)	Left Lateral Incisor (Control)
Model 2A	43.0° 44.0 44.0 44.0 46.0 45.0	69.5° 71.0 69.0 71.0 71.0 69.5	72.5° 74.5 60.0 73.0 75.0 73.0	44.5° 42.5 43.5 45.0 42.5 44.0
Mean	44.3	70.2	71.3	43.7
Model 2B	9.5 10.5 10.5 9.5 9.5 9.5 9.0	74.0 71.5 71.5 72.0 70.5 70.5 71.0	55.0 54.5 53.0 54.0 55.0 54.5 54.0	22.5 23.0 26.5 26.5 27.5 25.5 26.5
Mean	9.7	71.6	54.3	26.3
Model 2C	21.5 21.5 21.5 21.5 21.0 21.5	73.0 71.5 71.0 71.5 71.0 72.0	61.0 61.0 59.5 62.5 60.0 60.5	34.0 34.0 34.5 36.5 36.0 35.0
Mean	21.4	71.7	60.8	35.0
Model 2D	20.5 20.0 20.5 22.0 21.5 21.5	72.5 74.0 74.0 72.5 73.0 73.0	62.0 62.0 62.5 62.5 62.0 62.0	40.0 41.0 42.0 41.0 40.0 42.0
Mean	21.0	73.2	62.2	41.0

TABLE XI

ANGULATION OF MAXILLARY INCISORS TO MID-SAGITTAL PLANE

MONKEY #3

	Right Lateral Incisor (Rotated)	Right Central Incisor (Control)	Left Central Incisor (Rotated)	Left Lateral Incisor (Control)
Model 3A	27.0	69.0	68.5	32.0
	28.0	68.5	68.0	31.0
	27.0	69.5	68.5	30.0
	27.0	69.0	69.0	30.5
	27.5	68.0	68.0	30.5
	27.0	68.0	68.0	30.5
Mean	27.3	68.7	68.3	30.8
Left Central Rotated -washed in 70% alcohol -replaced in socket				
Model 3B	Crown Fractured At Neck	75.0	63.5	37.0
	-	75.0	62.0	37.0
	-	76.0	62.0	35.5
	-	76.5	61.5	35.0
	-	77.0	62.0	35.0
	-	76.0	62.0	35.0
Mean		75.9	62.2	35.1
Model 3C	-	74.0	Missing	28.0
	-	74.0	-	28.0
	-	74.0	-	28.0
	-	74.0	-	28.0
	-	74.0	-	28.0
	-	75.0	-	28.0
Mean	-	74.2	-	28.0
Model 3D	-	76.5	-	32.0
	-	76.5	-	31.5
	-	76.5	-	31.5
	-	76.5	-	33.0
	-	77.0	-	32.0
	-	75.5	-	33.0
Mean	-	76.4	-	32.2

TABLE XII

ANGULATION OF MAXILLARY INCISORS TO MID-SAGITTAL PLANE

MONKEY #4

	Right Lateral Incisor (Rotated)	Right Central Incisor (Control)	Left Central Incisor (Rotated)	Left Lateral Incisor (Control)
Model 4A	34.5	71.5	79.5	48.0
	34.5	72.0	79.5	46.0
	35.0	71.5	78.5	45.0
	34.5	70.5	79.0	45.5
	34.5	72.0	80.5	46.0
	35.0	71.0	80.0	44.5
	34.0	72.0	79.0	44.5
Mean	34.6	71.5	79.4	45.6
Model 4B	Missing	76.5	46.0	41.0
	Root Resorption,	75.0	46.0	40.0
	Tooth Exfoliated	74.0	45.0	43.5
	when band removed	75.5	46.0	37.5
		75.5	45.0	38.5
		73.5	45.0	42.5
Mean		75.0	45.5	40.5
Model 4C		No	Records	
Model 4D		84.0	43.5	44.5
		84.0	42.5	42.0
		84.0	42.5	43.0
		84.0	43.0	43.0
		84.5	44.5	44.0
		84.5	44.0	44.5
Mean		84.2	43.3	43.5

TABLE XIII

MEAN ANGULATION OF MAXILLARY INCISORS TO THE MID-SAGITTAL PLANE

MONKEYS (1, 2, 3, 4)

DENTAL CAST	ANGULATION OF TEETH IN DEGREES			
	Right Lateral Rotated	Right Central Control	Left Central Rotated	Left Central Control
MONKEY #1				
From Table IX	1A	36.2	65.5	69.4
	1B	22.7	69.2	48.8
	1C	30.7	73.2	Exfoliated
	1D	36.3	77.8	--
Monkey #2				
From Table X	2A	44.3	70.2	71.3
	2B	9.7	71.6	54.3
	2C	21.4	71.7	60.8
	2D	21.0	73.2	62.2
MONKEY #3				
From Table XI	3A	27.3	68.7	68.3
	3B	Fractured --	75.9	62.2
	3C	---	74.2	Exfoliated
	3D	---	76.4	---
MONKEY #4				
From Table XII	4A	34.6	71.5	79.4
	4B	Exfoliated	75.0	45.5
	No Records	---	---	---
	4C	---	84.2	43.3

TABLE XIV

RELAPSE OF TRAUMATICALLY ROTATED INCISORS

ANGULATION TO MID-SAGITTAL PLANE (DEGREES)

<u>INITIAL</u>	<u>After 12 Months</u>	
22.7	36.3	
9.7	21.0	
54.3	62.2	
45.5	43.3	
$-d = 8.75$	$sd = 35$	$sd^2 = 379.90$
$V(d) = 24.55$	$s-d = 2.48$	"t" test 3.53 df= 3 P = < 0.05

TABLE XV

ORTHODONTIC MOVEMENT OF CONTROL INCISORS

ANGULATION TO MID-SAGITTAL PLANE (DEGREES)

<u>INITIAL</u>	<u>AFTER 4 MONTHS</u>	
65.5	69.2	
36.5	36.3	
70.2	71.6	
43.7	26.9	
68.7	75.9	
30.8	36.1	
71.5	75.0	
45.6	40.5	
$-d = 5.40$	$sd = 43.2$	$sd^2 = 416.12$
$V(d) = 26.12$	$s-d = 1.81$	"t" test 2.98 df=7 P= < 0.05

TABLE XVI

RELAPSE OF CONTROL INCISORS

ANGULATION TO MID-SAGITTAL PLANE (DEGREES)

<u>INITIAL</u>	<u>AFTER 12 MONTHS</u>
69.2	77.8
36.3	40.9
71.6	73.2
26.9	41.0
75.9	76.4
36.1	32.2
75.0	84.2
40.5	43.5
$-d = 5.69$	$sd = 45.5$
$V(d) = 20.973$	$sd^2 = 405.59$
	"t" test 3.512 df = 7 P = < 0.01



Figure 1. Method of Intraperitoneal Injection of anaesthetic. Note the thick leather gloves for protection against bites. Rhesus monkeys may carry virus B which is fatal to man. B virus may be transmitted to man through a break in the skin caused by a bite.

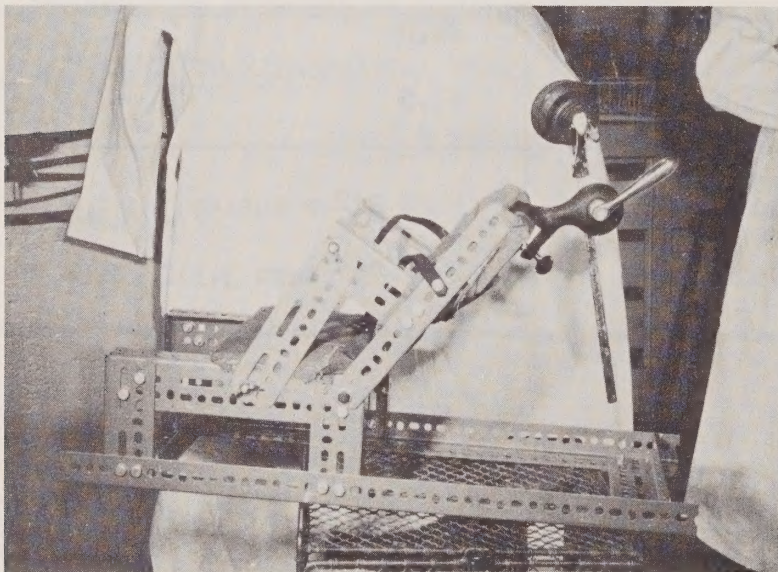


Figure 2. Lateral view of small dental chair specially designed for monkeys. The back of the chair may be tilted to the desired angulation by loosening wing nuts on the anterior posts of the arm rests.

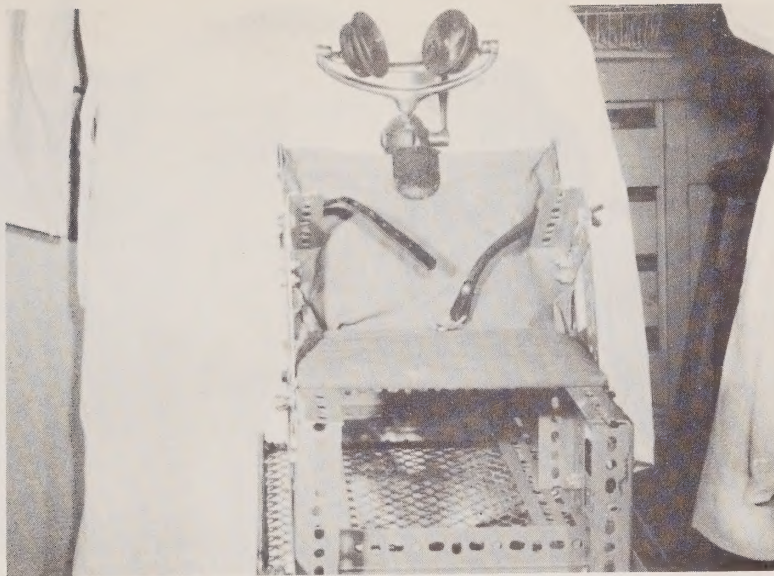


Figure 3. Anterior view of dental chair for monkeys.



Figure 4. Anaesthetized monkey has been placed in the chair and is ready for intubation.



Figure 5. The monkey has been intubated with a clear plastic tube. On each expiration, the tube fogged for a few seconds. The intermittent fogging proved very helpful in checking respiration. A large safety pin helped to stabilize the tube and hence reduce irritation to the trachea.



Figure 6. Technique for taking occlusal roentgenographs. All films were taken at an angulation of 65° to the maxillary occlusal plane, with a setting of 65 KV., 10 M.A. and exposure duration of 2 seconds.



Figure 7. Brass wire separation to open the contact points of the teeth to be banded.

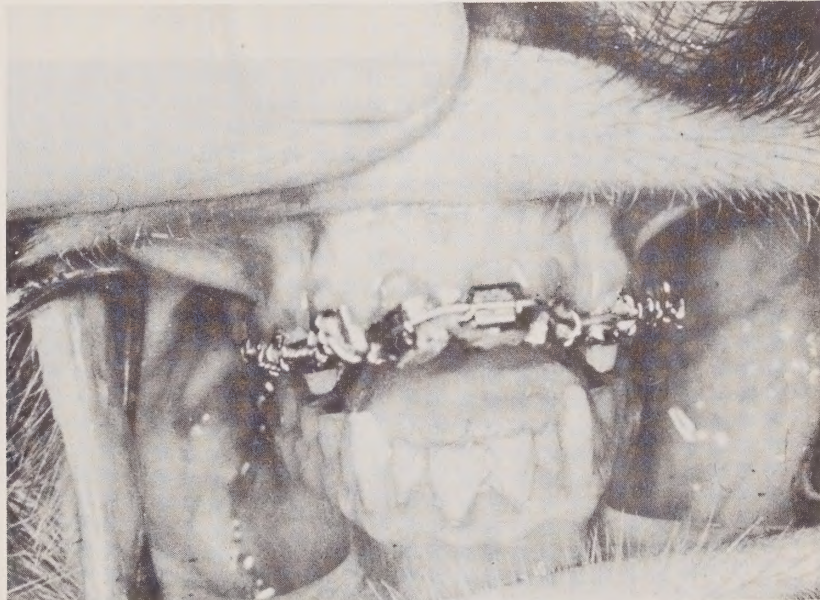


Figure 8. Anterior view to show the passive .020 round stainless steel labial arch ligated with .012 ligature wire to stabilize the rotated teeth until healing of the periodontium has occurred.

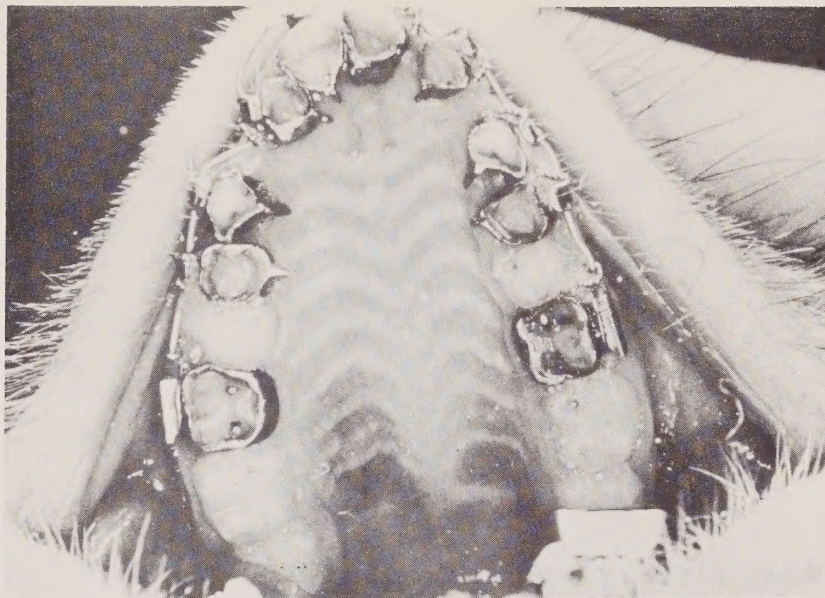


Figure 9. Occlusal view of passive .020 round stainless steel labial arch.

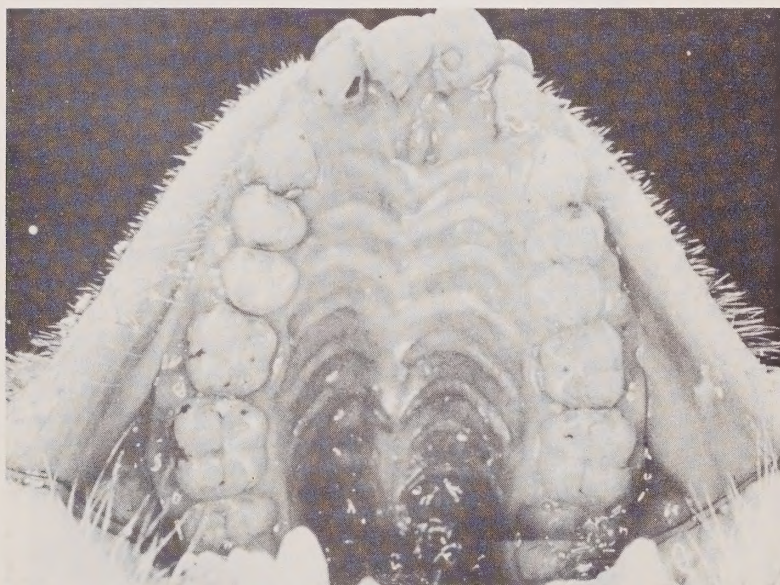


Figure 10. Occlusal view of the maxillary dental arch immediately after bands were removed. Note the rotation of the left central and right lateral incisors; also the pronounced gingivitis.

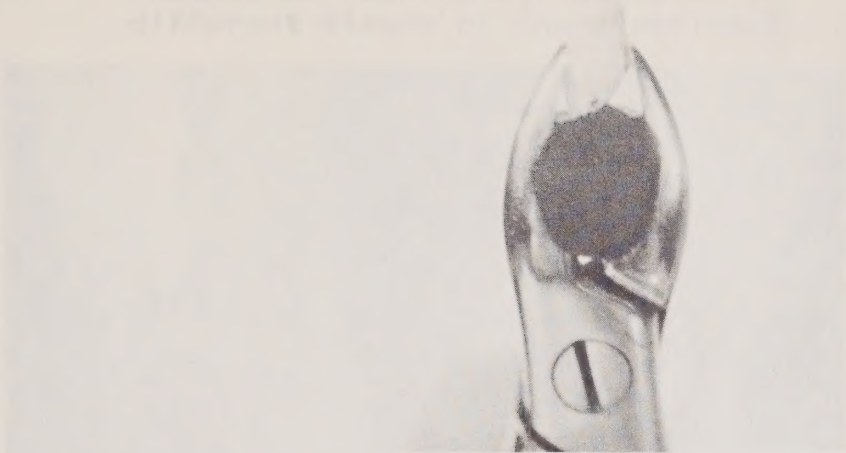


Figure 11. Picture to demonstrate how a small rubber cork was fitted within the confines of the forceps' beaks to prevent the tooth from popping through the beaks during the traumatic rotation.



Figure 12. Note the slight extrusion and altered axial inclination of the central incisor which occurred during the traumatic rotation.

FIGURE 13*

Photos. of roentgenograms taken at different stages of the experiment



Figure 13A - Corresponds to Model 1A - Fig. 17A
Just before traumatic rotation of left central
and right lateral incisors.



Figure 13B - Corresponds to Model 1B - Fig. 17B
Interval of 4 months after rotation. Bands
removed. Teeth have been rotated and periodontium
healed?

* See Table II for Interpretation of roentgenographs.

F I G U R E 13*



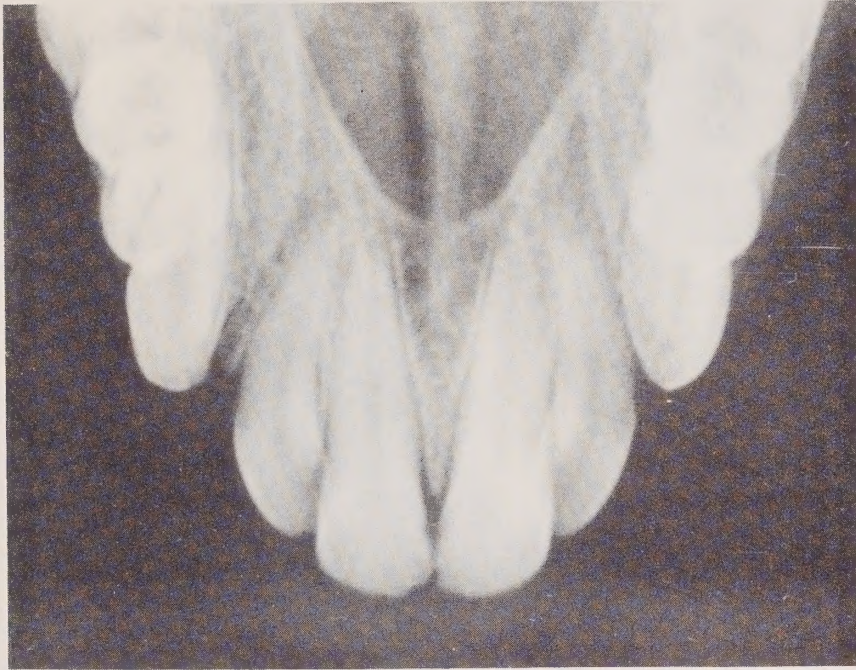
Figure 13C - Corresponds to Model 1C - Fig.17C
Interval of 13 months after rotation - progress
roentgenogram.



Figure 13D - Corresponds to Model 1D - Fig.17D
Interval of 21 months after rotation - taken
just prior to sacrifice.

F I G U R E 14*

**Photos of roentgenograms taken at
different stages of the experiment**



**Figure 14A - Corresponds to Model 2A - Figure 18A
Just before traumatic rotation of left central and
right lateral incisors.**



**Figure 14B - Corresponds to Model 2B - Figure 18B
Interval of 5 months after rotation. Bands just
removed. Teeth have been rotated and periodontium
healed?**

*** See Table III for interpretation of roentgenograms.**

F I G U R E 14*



Figure 14C - Corresponds to Model 2C - Figure 18C
Interval of 12 months after rotation. Progress
roentgenogram.

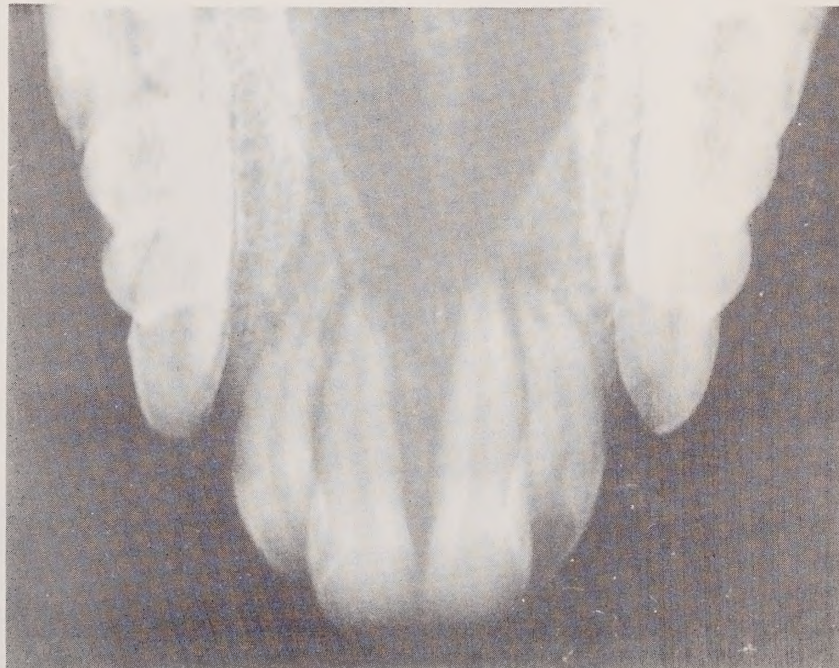


Figure 14D - Corresponds to Model 2D - Figure 18D
Interval of 20 months after rotation - taken just
prior to sacrifice.

FIGURE 15*

Photos. of roentgenograms taken at different stages of the experiment



Figure 15A - Corresponds to Model 3A - Fig. 19A
Just before traumatic rotation of left central
and right lateral incisors.

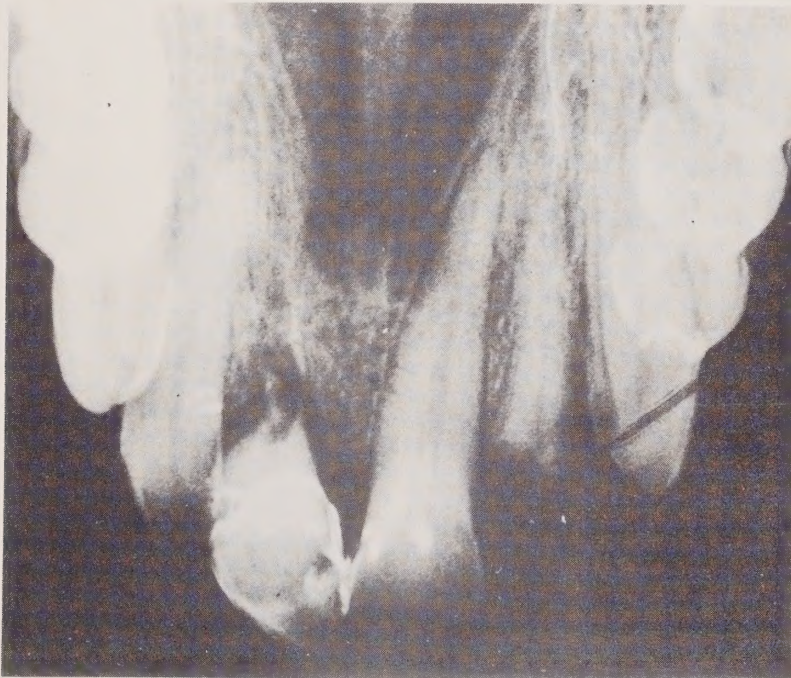


Figure 15B - Corresponds to Model 3B - Fig. 19B
Interval of 5 months after rotation. Bands
removed. Teeth have been rotated and periodontium
healed?

* See Table IV for interpretation of roentgenograms.

FIGURE 15



Figure 15C - Corresponds to Model 3C - Fig. 19C
Interval of 10½ months after rotation. Progress
roentgenograms.

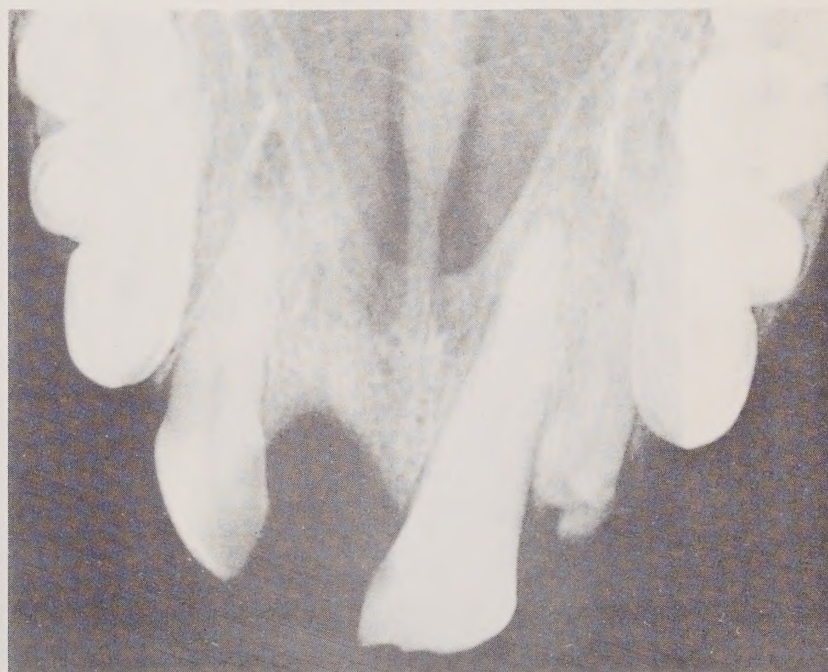


Figure 15D - Corresponds to Model 4C - Fig. 19D
Interval of 18 months after rotation. Taken
just prior to sacrifice.

FIGURE 16☆

Photos. of roentgenograms taken at different stages
of the experiment



Fig. 16A - Corresponds to Model 4A - Fig. 20A. Just before traumatic rotation of left central and right lateral incisors.

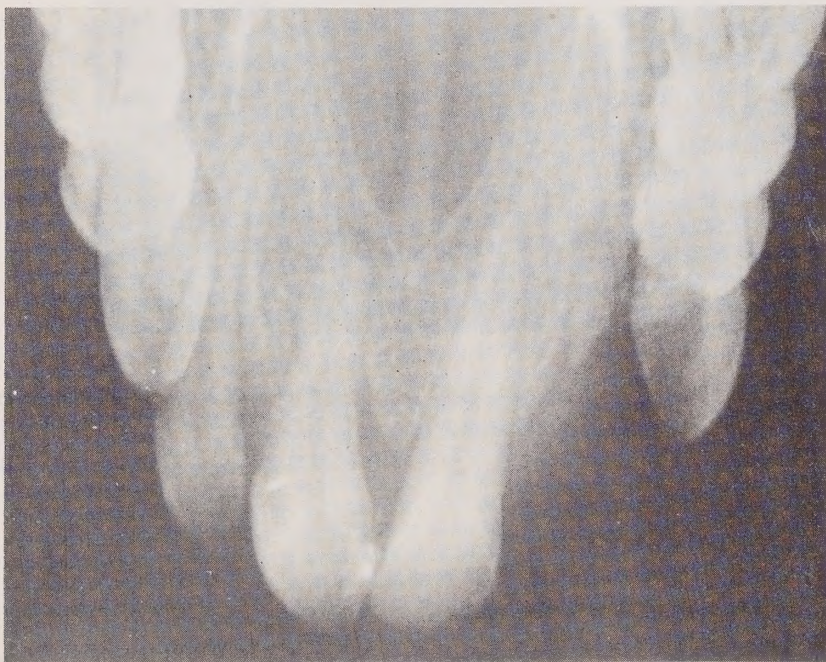


Fig. 16B - Corresponds to Model 4B - Fig. 20B. Interval of 6 months after rotation. Bands removed. Teeth have been rotated and periodontium healed.

FIGURE 16

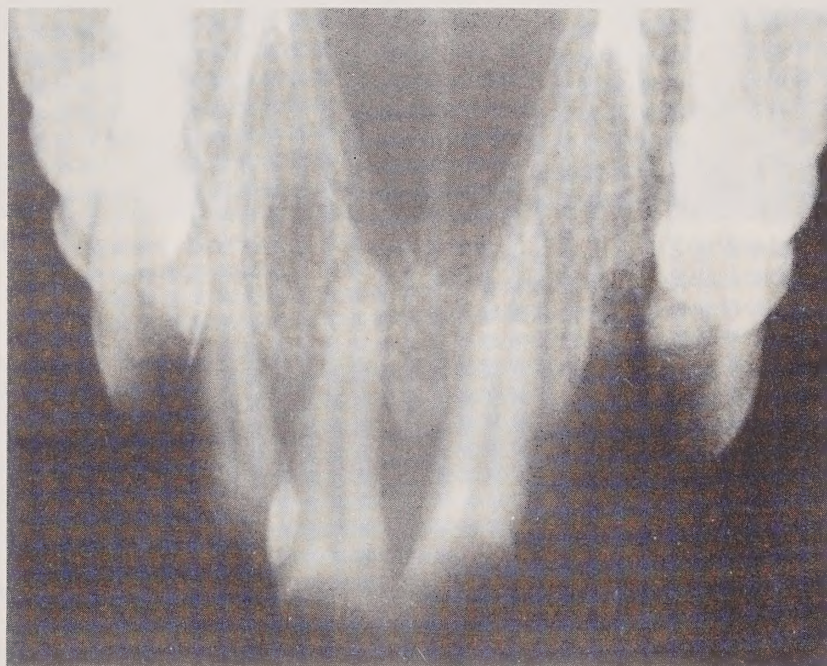


Figure 16D - Corresponds to Model 4D - Fig.20D
Interval of $17\frac{1}{2}$ months after rotation - taken
just prior to sacrifice.

Photos. of study models taken at
different stages of the experiment



Figure 17A - Taken before
traumatic tooth rotations



Figure 17B - Interval of 4
months after rotation. Bands
have just been removed.

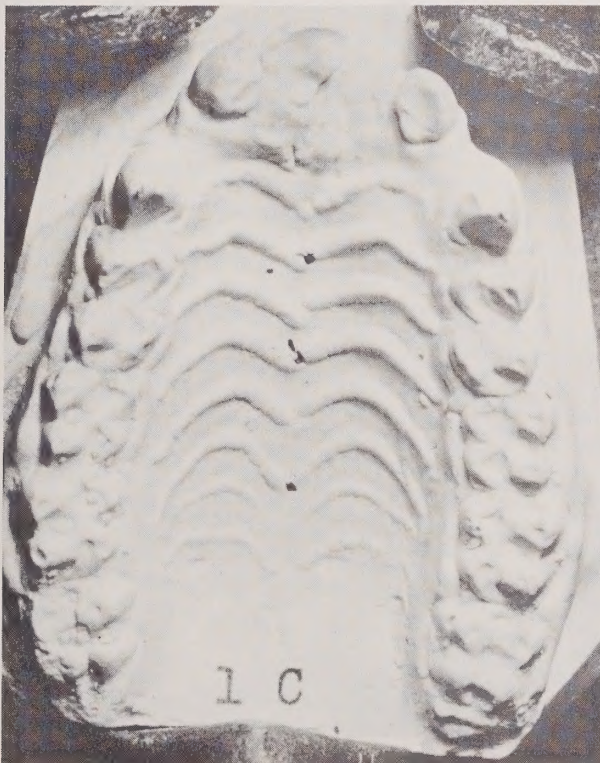


Figure 17C - Interval of
13 months after rotation.

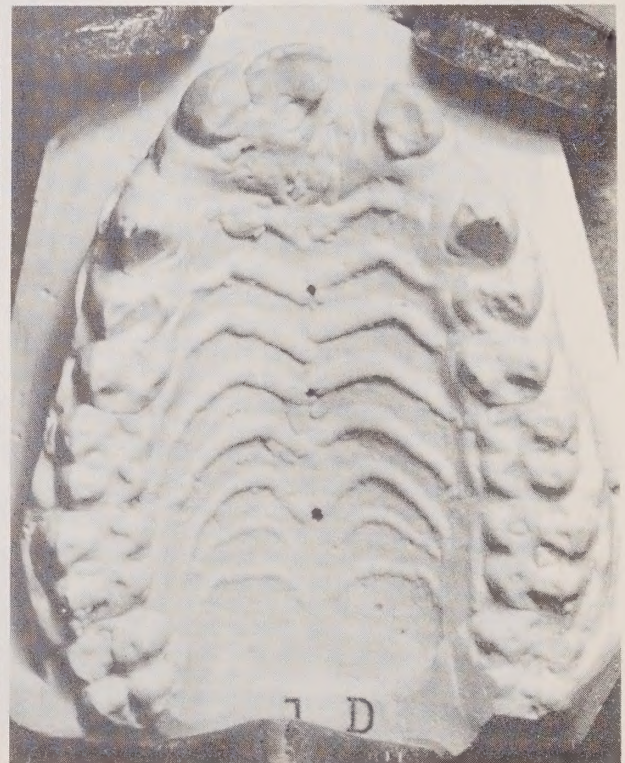


Figure 17D - Interval of 21
months after rotation. Taken
just prior to sacrifice.

* Figures 17A, 17B, 17C and 17D correspond to
centenogram Figures 13A, 13B, 13C & 13D respectively.

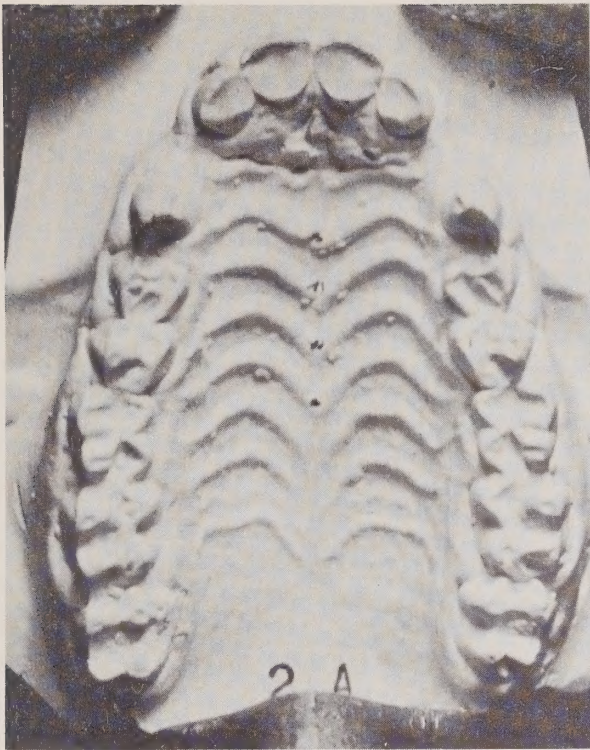


Fig. 18A - Taken before traumatic tooth rotations



Fig. 18B - Interval of 5 months after rotation. Bands have just been removed.

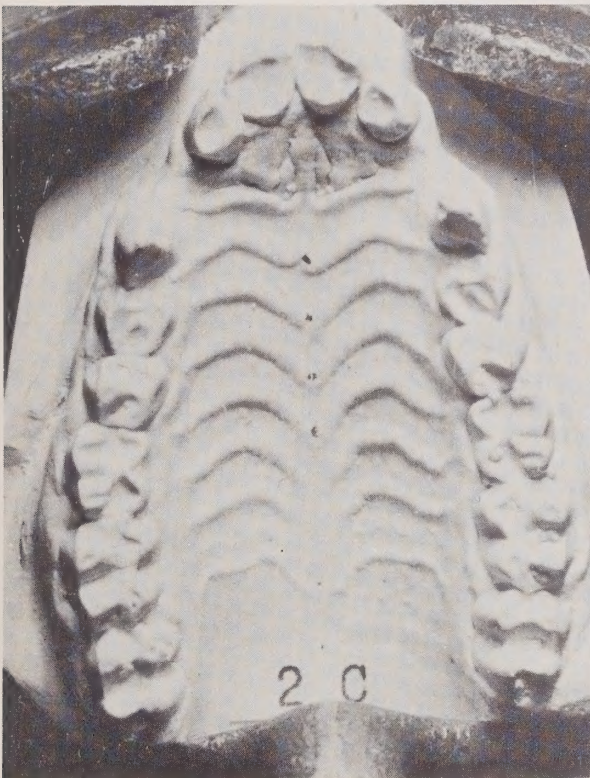


Fig. 18C - Interval of 12 months after rotation.



Fig. 18D - Interval of 20 months after rotation. Taken just prior to sacrifice

FIGURE 19*

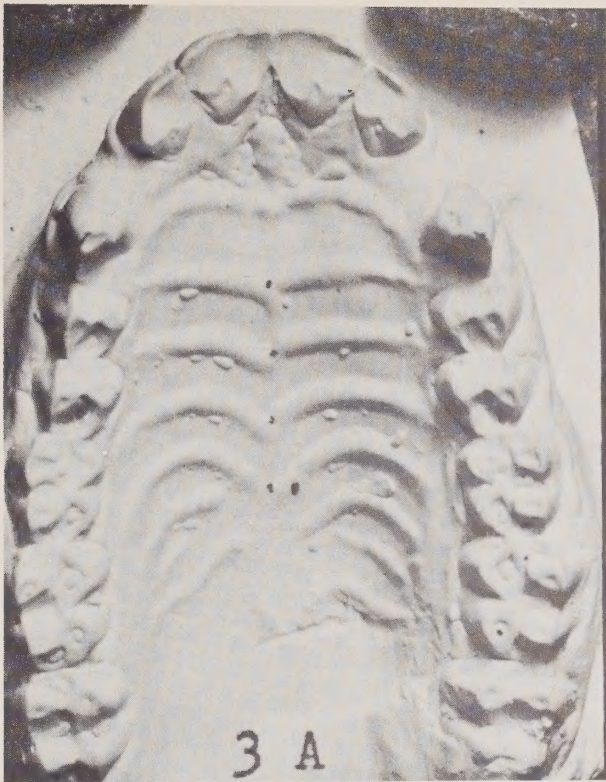


Figure 19A - Taken before traumatic tooth rotations



Figure 19B - Interval of 5 months after rotation. Bands have just been removed.



Figure 19C - Interval of 10½ months after rotation.



Figure 19D - Interval of 18 months after rotation. Taken just prior to sacrifice.

* Figures 19A, 19B, 19C and 19D correspond to roentgenogram Figures 15A, 15B, 15C & 15D respectively.

FIGURE 20*



Figure 20A - Taken before traumatic tooth rotations

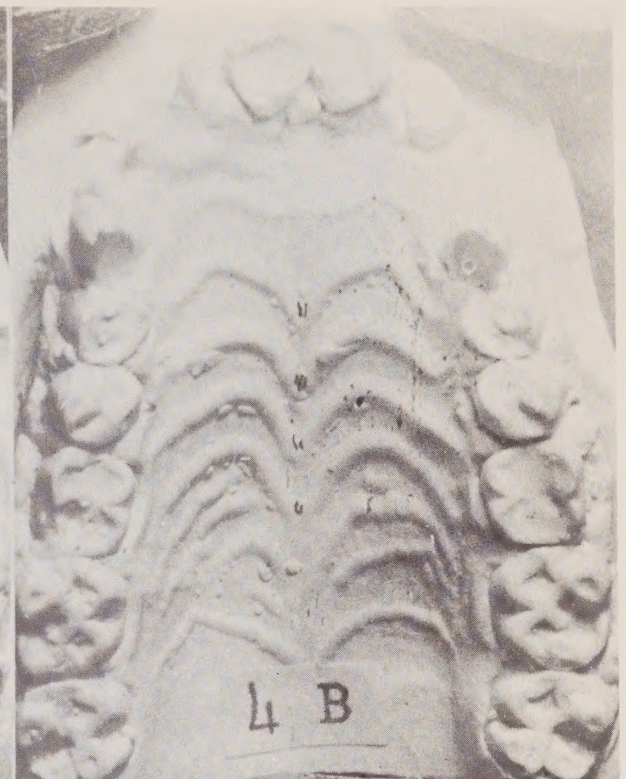


Figure 20B - Interval of 6 months after rotation. Bands have just been removed.



Figure 20D - Interval of $17\frac{1}{2}$ months after rotation. Taken just prior to sacrifice.

* Figures 20A, 20B and 20D correspond to roentgenogram Figures 16A, 16B and 16D respectively.

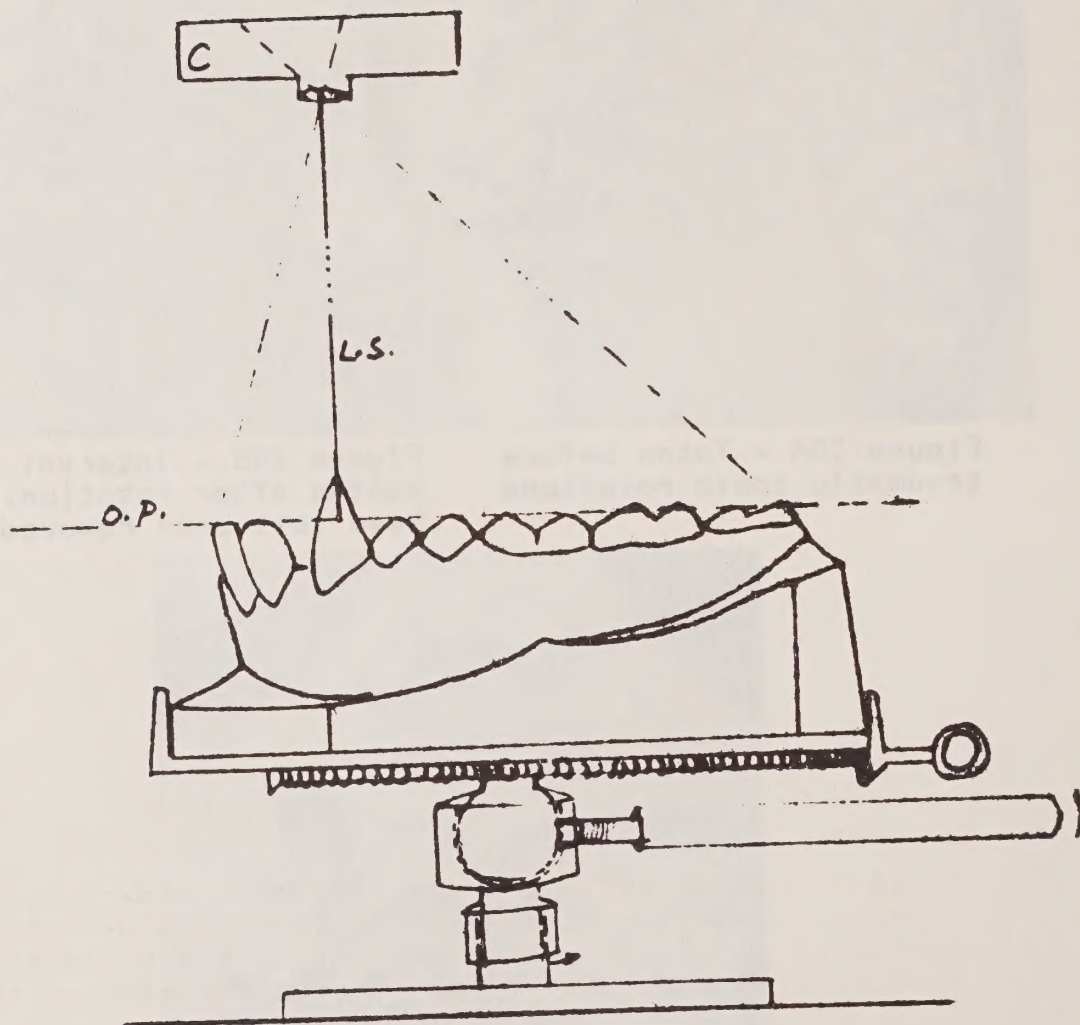
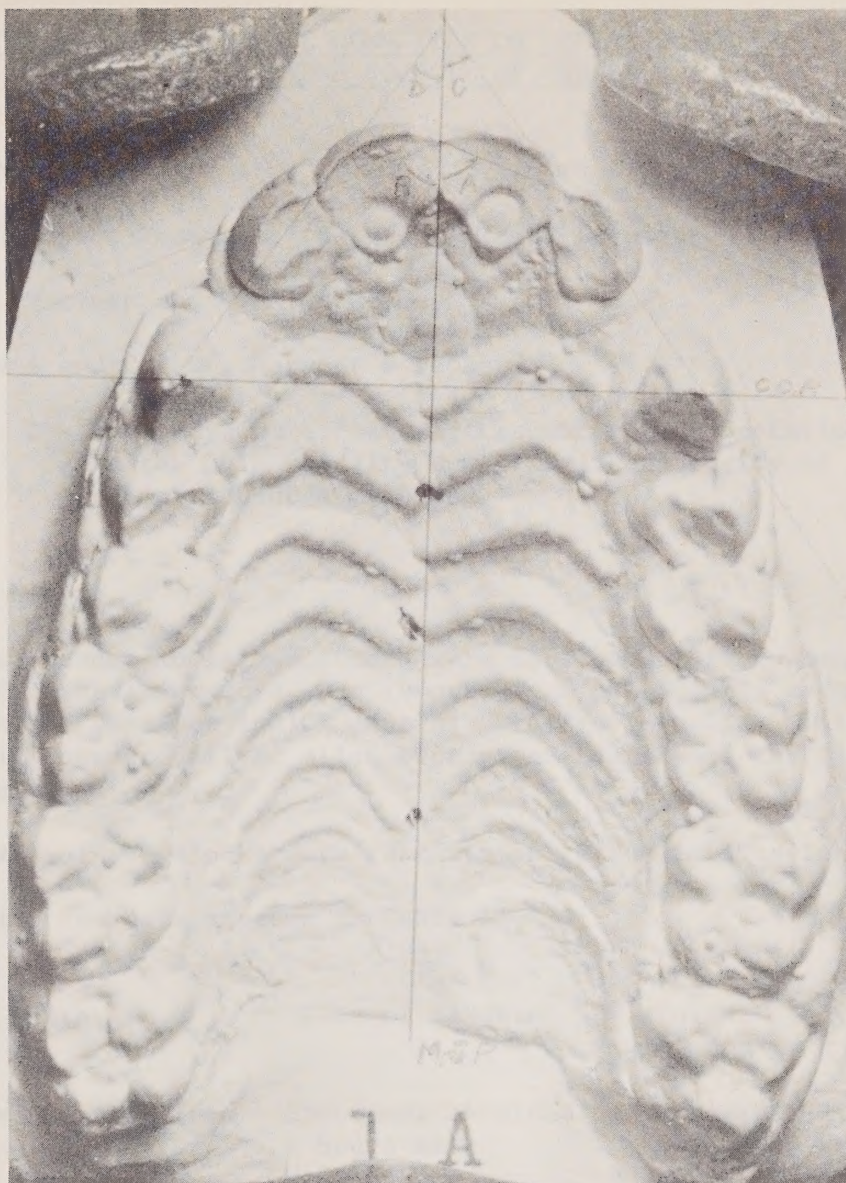


Fig. 21

Diagrammatic sagittal view of method of photographing dental casts.

C - camera L.S. - Lens to subject distance.
 O.P. - Horizontal line contacting incisal edge of central incisor and tips of mesial-buccal cusps of first molars.

FIGURE 22*



- A - Angulation of left central incisor to mid-sagittal plane.
- B - Angulation of right central incisor to mid-sagittal plane.
- C - Angulation of left lateral incisor to mid-sagittal plane.
- D - Angulation of right lateral incisor to mid-sagittal plane.

M.-S.P. - Mid-sagittal plane - determined by selecting three points approximately 1.5 cm. apart on the median raphe of the palate. The identical three points originally chosen were used throughout for all the measurements on the same animal.

C.C.P. - Canine to canine plane - determined by joining the tips of the cusps of the canines. This plane was used in photographing the models. Cross hairs on the focusing ground glass were superimposed on the mid-sagittal plane and the tips of the cusps of the right and left canine.

* Actual enlargement used in measuring the angulation of the incisors to the mid-sagittal plane of the palate. Six measurements were made for each tooth and then averaged to reduce errors in measurement (See Table IX).

January 11, 1961.

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH
NATIONAL RESEARCH COUNCIL OF CANADA

Project: NO.DA.69

Subject: The Study of Traumatic (Surgical) Tooth Movement.

Grantees: R. D. Haryett and K. A. McMurchy

Purpose: Phase A and C - Dr. Haryett's report

Phase B - to study the effects of traumatic tooth rotations
on the pulp vitality and ultimate integrity of
the periodontal membrane.

Histological Technic

Four monkeys and one individual tooth were received. Blocks containing the upper central and lateral incisors were removed from the animals as soon after death as possible and were put in 10% neutral buffered formalin. Pulpes were exposed with a high speed drill to allow better penetration of the fixative and embedding materials.

Roentgenograms of the blocks were taken prior to decalcification.

Pertinent fixation data appears in Table 1.

Monkey	Death	Time of fixation	Pulpes Exposed	Condition of Teeth
1	Killed with chloroform	Specimens obtained 2 hours after death.	48 hours after death.	Right central loose. Probably as a result of a blow. Left central missing.
2	Killed with chloroform	Specimens obtained 1½ hours after death.	3 hours after death.	All teeth firm. All teeth present.
3	Died of overdose of nembutal.	Specimens obtained 1½ hours after death.	3 hours after death.	Left central missing. Right lateral crown missing.
4	Died of unknown causes.	Head in refrigerator for 12 hours, in 10% formalin for 18 hours before specimen was obtained and put in fresh formalin.	18 days after death - after decalc.	Left central discoloured. Right lateral missing at death but had been processed previously.

Specimens were dehydrated with alcohols, embedded in paraffin and cut perpendicular to the long axes of the teeth so that controls and rotated teeth would be in the same sections.

Sections were labelled A, B, C, D or E, depending on the level of cutting as in Fig.1.



E
D
C
B
A

Fig.1 Monkey 2



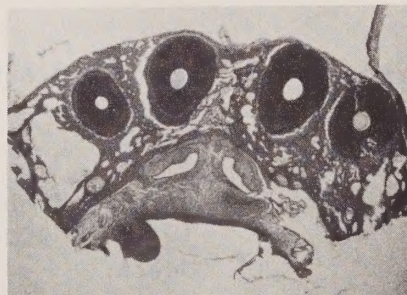
M.2E



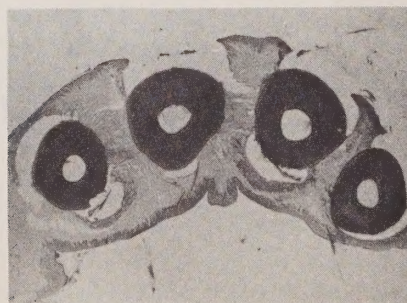
M.2D



M.2C



M.2B

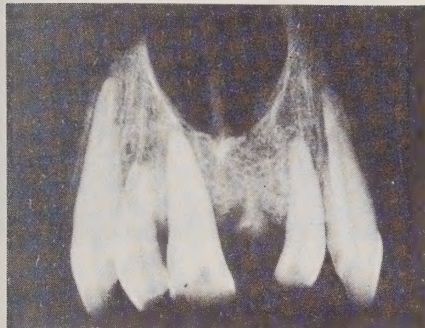


M.2A

Results

Monkey One

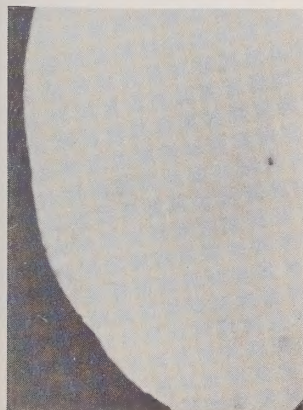
A. Roentgenogram



R M.1 L

Comments - the right lateral and left central are abnormal in appearance. These were the rotated teeth.

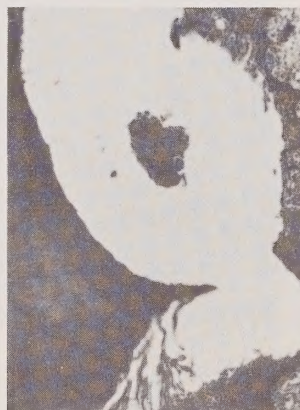
B. Condition of Pulp



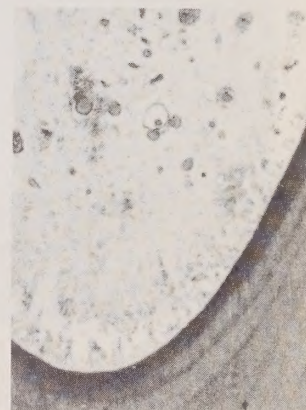
RL



RC



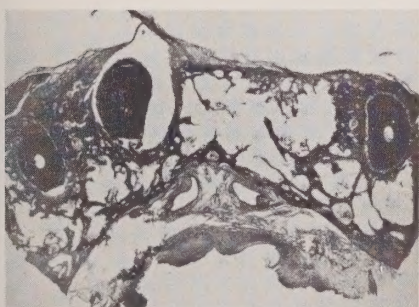
LC



LL

Comments - The pulps of the control teeth, RC and LL are normal in appearance. Predentin and odontoblasts are present. The pulp of the rotated teeth RL and LC have undergone liquefaction necrosis and the predentin layer has calcified. The LC had fractured during the rotation operation and the apical portion shows considerable resorption.

C. Periodontal Membrane



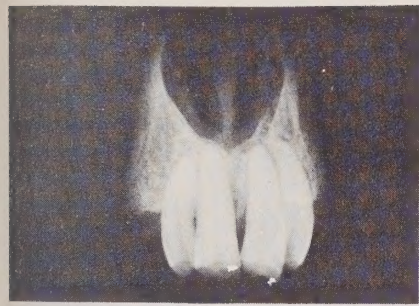
R M.1C L

Observations:-

- RL - Resorption and inflammation of periodontal membrane.
- RC - Torn periodontal ligament due to blow just before death.
- LC - Resorption.
- LL - Normal periodontal membrane.

Monkey Two

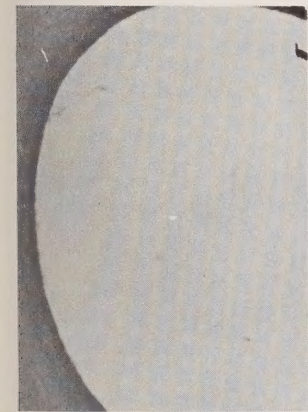
A. Roentgenogram



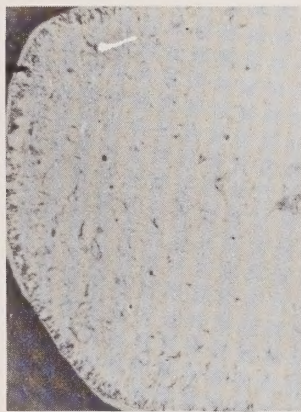
Observations - there are radiolucent areas at the apices of RL and LC. These are the teeth which were surgically rotated.

R M.2 L

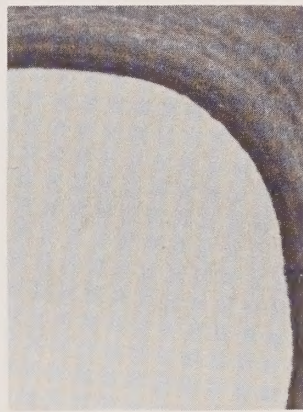
B. Pulp



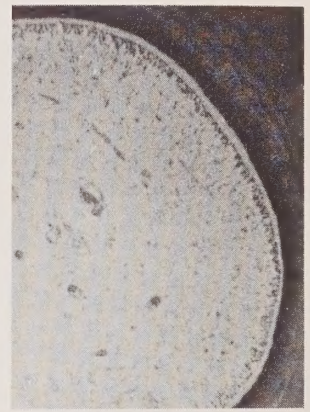
RL



RC



LC

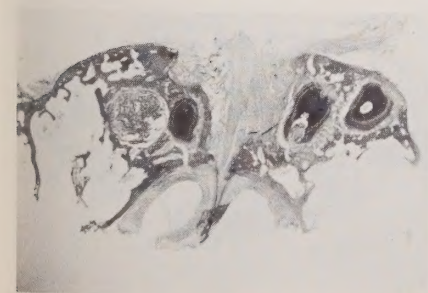


LL

Histopathological Observations

The pulps of the control teeth, RC and LL are normal with distinct odontoblasts and predentin layers. The pulps of the rotated teeth, RL and LC have undergone liquefaction necrosis and the predentin layer has become mineralized.

C. Periodontal Membrane



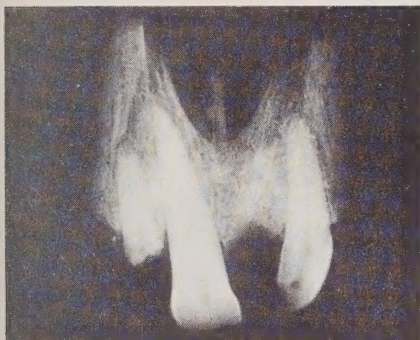
Observations:-

- RL - Epitheliated granuloma.
- RC - Normal periodontal membrane.
- LC - Resorption and granuloma at apex.
- LL - Some labial resorption.

R M.2D L

Monkey Three

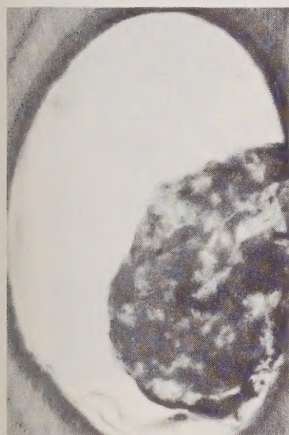
A. Roentgenogram



R M3 L

Observations - Only the root of the right lateral is present and it appears to have undergone resorption at the apex. The other experimental tooth, the left central, is missing.

B. Pulp



RL



RC



LL

Observations - The rotated right lateral shows resorption, loss of pulp tissue and mineralization of the predentin layer. The control teeth, RC and LL have odontoblasts and predentin but show some congestion and small hemorrhages which may be associated with the abnormal death of this animal due to an overdose of nembutal.

C. Periodontal Membrane



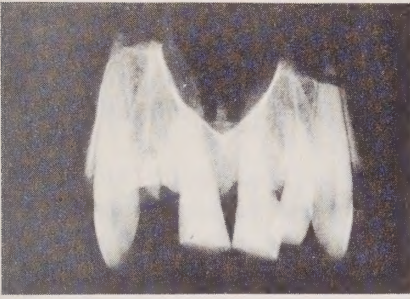
R M.3C L

Observations:-

- RL - Abscess on lingual.
- RC - Normal periodontal membrane.
- LC - Absent.
- LL - Normal periodontal membrane.

Monkey Four

A. Roentgenogram



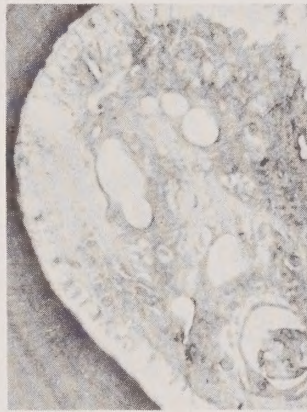
Observations - Only the apex of the right lateral remains. The left central has a wide periodontal membrane and the root appears to have undergone resorption. These are the rotated teeth.

R M.4 L

B. Pulp



RL



RC



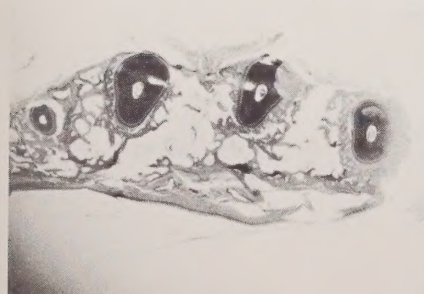
LC



LL

Observations - The rotated RL had fractured during the surgical rotation and the incisal two thirds had exfoliated. The remaining apical portion shows extensive resorption, calcification of predentin and debris and micro-organisms in the root canal. The rotated left central was discolored and shows reticular atrophy of the pulp. The predentin has undergone mineralization only in a few areas and some odontoblasts are identifiable. Large vessels containing thrombi are present. The control teeth RC and LL have odontoblasts and predentin but show extreme congestion of blood vessels, hemorrhage and thrombus formation. This is probably the result of circulation disturbances associated with the premature death of this animal from unknown causes.

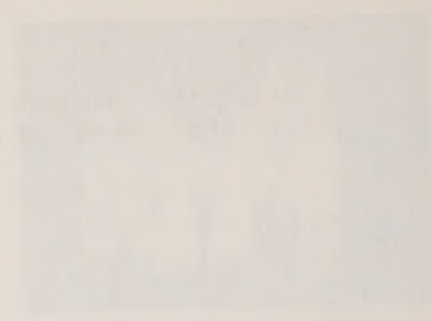
C. Periodontal Membrane



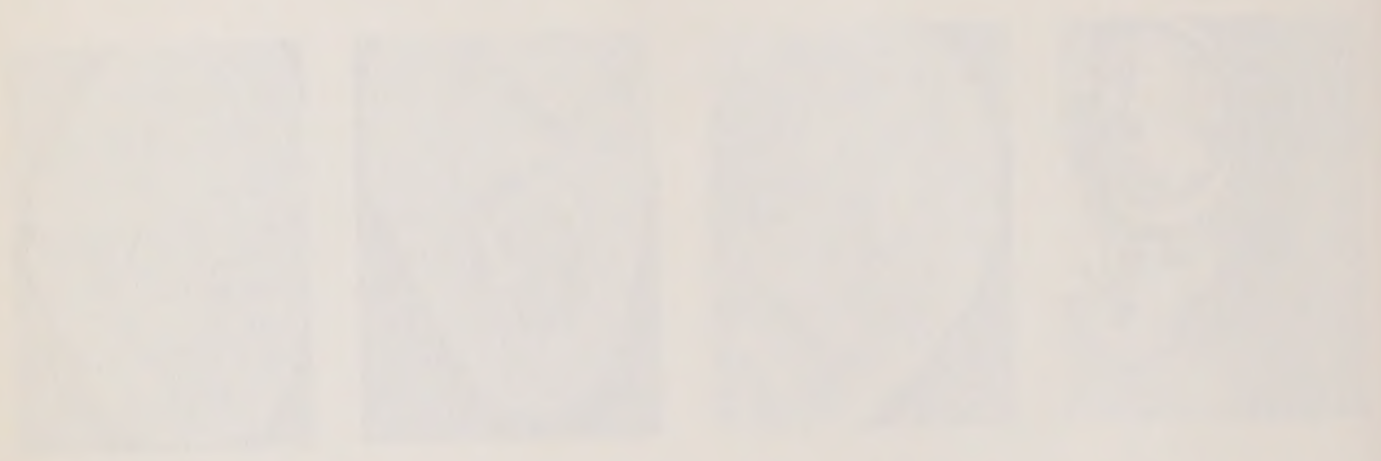
Observations:-

RL - Resorption and inflammation.
 RC - Normal periodontal membrane.
 LC - Extensive disto-labial root resorption.
 LL - Distal root resorption.

R M.4C L



The following is a list of the items
 found in the room. The list is not
 complete, but it is a good start.
 The items are listed in the order
 in which they were found.



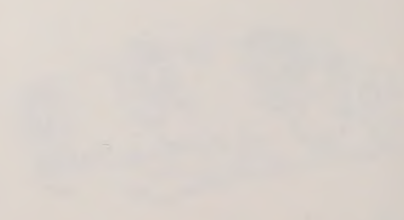
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 The items are listed in the order
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Conclusions

1. The experimental rotation resulted in the death of all of the pulps of the rotated teeth, while all the controls remained normal.
2. The pulp deaths resulted in inflammatory reactions in the apical periodontal membrane. These reactions tended to be acute if the tooth was fractured and chronic if the tooth was intact.
3. Resorption of the roots was extensive in the experimental teeth and minimal in the control teeth.
4. Gingivitis was prevalent around both experimental and control teeth but was more severe around the experimental teeth. The epithelial attachment had often receded apically in the experimental teeth.
5. In many areas on the roots of the experimental teeth, the periodontal fibers had been reattached and reconstructed so as to appear completely normal.
6. It would appear that most of the resorption and inflammatory reaction was associated with the fact that the pulps become necrotic. This suggests the possibility of minimizing these reactions by doing pulp extirpations and canal obturation on the teeth prior to their rotation.

Respectfully submitted,

K. A. McMurchy

K. A. McMurchy, D.D.S.

F. James Marshall, D.M.D., M.B.
Associate Professor
Endodontology & Oral Histology

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Respectfully submitted,

K. A. McNulty

K. A. McNulty, D.D.S.

APPENDIX I

PROGRESS REPORT DA-83

1. This Grant was activated by The University of Manitoba April 27th, 1960.
2. A Radio-isotope Laboratory was designed and construction authorized June 1960. Construction was completed sufficiently to operate December 22, 1960.
3. The necessary Radio-isotope monitoring and storage equipment has been purchased along with some general laboratory supplies.
4. Preliminary work has been done with post mortem dog material (teeth in situ in freshly dissected dog jaws) to familiarize the operators with dog tooth anatomy. These studies have helped to modify and direct the endodontic techniques required for the in vivo procedures.
5. Several dogs have been obtained and are being prepared for treatment in the near future as outlined in the original submission.
6. Further delays to this project are not anticipated either for reasons of construction or licensing requirements of the Atomic Energy Commission and the study should now proceed without interruption to completion in the next year.
7. The initial budgetary requirements for this project have been adequate and additional funds are not requested for the coming year, as the balance on hand is deemed adequate.

Findings: -

- 1). Apparent initial: F. James Marshall, D.M.D., M.S.
Associate Professor
Endodontology & Oral Histology

2). The formation of a fibrous graft which is more extensive in the infected rate.

3). Considerable activity of the bone ends in some of the specimens but in general there was little or no activity.

F. James Marshall

THE USE OF AN ANORGANIC BONE GRAFT FOLLOWING PARTIAL
MANDIBULAR RESECTION IN THE RAT

Progress Report

Project DA-74

Review of Procedure:

Following exposure of the mandible via a submandibular approach and resection of a portion of the body of the mandible an anorganic bone graft was ligated into position with chronic gut sutures. In the control animals a "bakelite" graft was inserted to maintain the position of the resected bone ends.

Our chief problem continues to be one of infection at the surgical site. Culture and sensitivity studies have been done and Ilotycin still remains the antibiotic of choice.

Summary of Histological Findings:

Study of the histological sections reveal the following findings: -

- 1). Apparent initial infiltration of the anorganic graft with osteoblastic and osteoclastic cells.
- 2). The formation of a fibrous capsule about the anorganic graft which is more extensive in the infected rats.
- 3). Considerable activity of the bone ends in some of the specimens but in general there was little or no activity.

4). In many of the specimens there was interposing of muscle and fatty tissue between the encapsulated graft and the bone ends.

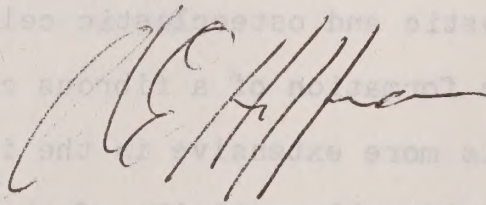
5). In many of even the older specimens, fragments of host bone exhibited encapsulation and chronic inflammatory cell activity with what appeared to be a rather slow osteoclastic action.

Evaluation of Histological Findings:

Further histological evaluation indicates at this time that the anorganic bone graft is being rejected by the host as evidenced by the formation of a fibrous capsule about the graft and the comparative inactivity of the cut ends of the bone.

In some of the specimens there is, however, cellular activity in the lacunae of the graft suggestive of osteoblastic and osteoclastic activity. This would appear to warrant further investigation, but in view of existing circumstances, I prefer not to seek further assistance but to carry on the investigation on my own in the meantime.

Respectfully submitted



A. E. Hoffman, D. D. S.

L. F. BELANGER

December 7th, 1960.

FINAL REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCHProject DT 52: THE INTIMATE MECHANISM OF MINERALIZATION:Parts 13 and 14: The intimate effect of osteolathyrism:

(1) Autoradiographic Studies of sulfated mucopolysaccharide metabolism in cartilage of osteolathyric Rats and Chicks, Leonard F. Bélanger, Proc. Soc. for Exp. Biol. and Med., 99, 605-607, 1958.

Summary: In lathyric rats and chicks, the in vivo sulfate fixation per individual cell was equal or slightly higher than that of normal animals. Since there were more active cells in the hypertrophic plate of the lathyric rat, the overall sulfur uptake by cartilage was apparently greater. In 7 day lathyric chicks, the plate was not hypertrophied but the sulfate uptake seemed slightly greater. In vitro Ca^{45} uptake by demineralized cartilage of the upper extremity of the tibia was considerably less than that of the lower extremity and also less than that of controls, indicating a more rapid utilization or turnover.

(2) Observations on the manifestations of osteolathyrism in the chick. Leonard F. Bélanger, J. Bone and Joint Surg., 41 B, 581-589, 1959.

Summary: Severe osteolathyrism has been induced in chicks of different ages by a diet containing 50 per cent seeds of *Lathyrus odoratus*. In these chicks, most

of which became paraplegic after seven days, a meningeal tumour, articular and bony deformities, spontaneous fractures and osteoporosis have been observed. In cartilage the lesion involves depletion of both neutral and acidic polysaccharides. The primary effect on bone consists of changes in the osteoblasts and in the osteocytes involving cytoplasmic granulation and vacuolation, "mineralization" and eventual disintegration of the cells. These events are followed by osteoporosis, abnormal mineral deposits in the marrow spaces and reactive callus-like activation of the periosteum. The role of the osteoblast and of the osteocyte in mineral transit and deposition is reconsidered.

Part 15: The effect of Vitamin D on S^{35} uptake and turnover at the epiphyseal plate of rachitic chicks:

(1) Autoradiographic observations on the uptake and transit of radiosulfate in the epiphyseal cartilage of rachitic chicks and the effects of Vitamin D, Leonard F. Bélanger and B. B. Migicovsky, submitted to Can. J. Biochem. and Physiol.

Summary: An autoradiographic survey of $S^{35}O_4$ in rachitic and vitamin D treated chicks has revealed:

(1) a slow uptake and turnover by the articular cartilage; (2) a major uptake by the primary small and large cells of the conjugation cartilage; (3) a general decrease of sulfate synthesis in low Ca-vitamin D deficiency; (4) stimulation of sulfate synthesis 18 to 24 hrs. after oral administration of vitamin D₂; (5) accelerated turnover at

the level of the small cells, in presence of vitamin D; (6) slow turnover in the zone of large cells and growth transit of the labelled matrix; (7) total replacement of the zone of large cells within 5 days; (8) absence of transit of sulfate from the zone of small cells to the underlying zone of large cells.

(2) Early changes at the epiphysis of rachitic chicks, following administration of Vitamin D. Leonard F. Bélanger and B. B. Migicovsky, J. Exp. Med., 107, 821-828, 1958.

Summary: One day old chicks were fed a vitamin D-deficient diet for 3 weeks. Some were then given an oral dose of vitamin D and sacrificed at various times thereafter. The tibiae were x-rayed; demineralized sections were stained for neutral polysaccharides and sulfated mucopolysaccharides; other specimens were ashed; and others still were autoradiographed to determine their uptake of Ca^{45} in vitro.

Mineralization reappeared in the treated animals after 2 days, along with new P.A.S.-positive spicules.

Earlier, the immature cartilage rapidly matured morphologically. At the same time, the rachitic matrix, highly concentrated in cation binder, presumably chondroitin sulfate, lost this material progressively.

(3) Comparative effects of vitamin D, calcium, cortisone, hydrocortisone, and norethandrolone on the epi-

physeal cartilage and bone of rachitic chicks. Leonard F. Bélanger and B. B. Migicovsky, *Develop. Biol.*, 2, 329-342, 1960.

Summary: Rachitic chicks were treated with vitamin D, calcium phosphate, cortisone, hydrocortisone, norethandrolone, or a combination of these factors. Vitamin D, norethandrolone, and calcium promote the maturation of cartilage matrix. Cortisone and hydrocortisone inhibit this process. Vitamin D and calcium promote the maturation of subepiphyseal bone cells and matrix. Norethandrolone promotes the growth of immature bone cells which secrete a small amount of matrix. Maturation of subepiphyseal bone is produced by the association of norethandrolone with calcium phosphate. The effects of vitamin D, the biochemistry of cartilage matrix maturation and its role in mineralization are discussed.

Part 16: The effect of hypophysectomy and growth hormone in S^{35} methionine and H^3 thymidine uptake at the epiphyseal plate of growing rats: The collaboration with U. C. Berkeley was discontinued due to the illness of Professor Asling. This project however was initiated with Dr. Pierre Bois. Hypophysectomized rats were treated with Growth Hormone, growth hormone + Amino-aceto-nitrile (a potent lathyrogenic agent) and the H^3 thymidine uptake was compared to that of hypophysectomized animals and

normal controls. The AAN-growth hormone group showed loss of ground substance and marked fibrillar appearance of the cartilage. On the other hand, this tissue and also the growing portion of new dentine at the root have shown the largest rate of H^3 thymidine uptake, indicating that the nuclear metabolism is not adversely affected by the AAN.

Other projects:

(1) Persistent toluidine blue metachromasia. Leonard F. Bélanger and A. Hartnett, J. Histochem. and Cytochem., 8, 75, 1960.

(2) A comparison between different mucopolysaccharide stains as applied to the epiphyseal chick cartilage. Leonard F. Bélanger and B. B. Migicovsky, in press with J. Histochem. and Cytochem.

Summary: The localization of the periodic acid-Schiff reaction, that of toluidine blue metachromasia, of Alcian blue and the Hale reaction does not coincide in the epiphyseal cartilage of the chick. The iron-adsorption pattern of the Hale reaction corresponds to that of Ca^{45} in vitro uptake and represents probably a chelating fraction of cartilage or unbound chondroitin sulfate. Toluidine blue metachromasia is less specific and may well represent collagen-bound as well as unbound sulfated mucopolysaccharides. The PAS stained material predominates in the articular cartilage and in the zone of large cells of the conjugation cartilage. Its intimate distribution matches

the pattern of high density revealed by alphasradiography. It is probably a protein-bound high polymer. The intracellular and immature matrix distribution of Alcian blue points to its staining of a precursor component. The existence of an interlacunar network of PAS and Alcian blue stained fibers in the articular cartilage, has been confirmed.

(3) Alphasradiography: A simple method for determination of mass concentration in cells and tissues. Leonard F. Bélanger, J. of Biophys. and Biochem. Cytol., 6, 197-202, 1959.

Summary: A simple apparatus for the production of contact microradiographs with the help of polonium alpha source and nuclear emulsion plates is described. This apparatus best adapted for soft tissue and low grade mineralization studies offers advantages as to resolution, geometry of specimen as well as ease of operation and cost.

(4) The new method of alpha-ray microradiography applied to the study of chick cartilage. Leonard F. Bélanger and B. B. Migicovsky, Trans. Roy. Soc. of Canada, LIII, Sec. V, 21-28, 1959.

Summary: The new method of alpha ray microradiography ("alphasradiography") utilizing a Polonium plated source has been successfully applied to a survey of demineralized chick epiphysis under various conditions. The normal bird shows variable density in the articular zone;

low density in the zone of small cells and high density in the zone of large cells, bone spicules, perichondrium and articular surface. Rickets and cortisone lower the density of the articular and conjugation cartilage.

Vitamin D and Nilevar produce an increase of density in the articular and conjugation portions of the epiphysis.

In the conjugation cartilage, the response is greater in the zone of small cells with vitamin D and in the zone of large cells with Nilevar. In regards to matrix, comparative observations indicate that low density is associated with predominance of chondroitin sulfate, while high density is probably related to an abundance of collagen fibers. High density of matrix follows blood vessels. Areas of low density are poorly vascularized except in cortisone-treated birds.

(5) Development, structure and composition of the otolithic organs of the rat. Leonard F. Bélanger, reprinted from Calcification in Biological Systems, 1960, by the Amer. Assoc. for the Advancement of Science, Washington, D. C.

Summary: The otoconiae are heterogeneous crystalline bodies; they grow by coalescence and fusion of smaller particles and subsequent lamellar apposition.

They contain calcium, possibly phosphorus, also neutral and acidic sulfated mucopolysaccharides; all these constituents are concentrated peripherally. They pick up Ca^{45} from the environment more rapidly in vitro than

low density in the zone of small cells and high density in vivo in comparison with adjacent bone. The otolithic membrane contains also neutral and acid sulfated mucopolysaccharides but in different proportions; it contains no mineral salt.

The zone of small cells with vitamin B and in the zone of large cells with vitamin B. In regards to matrix, comparative observations indicate that low density is associated with predominance of chondroitin sulfate, while high density is probably related to an abundance of collagen fibers. High density of matrix follows blood vessels. Areas of low density are poorly vascularized except in collagen-pressed birds.

(2) Development, structure and composition of the otolithic organs of the rat. Leonard F. Halasz, reported from California in Biological Systems, 1960, by the Amer. Assoc. for the Advancement of Science, Washington, D. C.

Summary: The otoliths are heterogeneous dry-falline bodies; they grow by coalescence and fusion of smaller particles and subsequent lamellar apposition. They contain calcium, possibly phosphate, also neutral and acidic sulfated mucopolysaccharides; all these constituents are concentrated peripherally. They pick up Ca²⁺ from the environment more readily in vitro than

APPENDIX L

PROGRESS REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

National Research Council of Canada

Grant: DT-59

Grantee: D. Harold Copp, M.D.

Assistant: A. P. Suiker

Department of Physiology, University of British Columbia.

Report of work to: December 31, 1960.

INTRODUCTION

When animals are restricted to a diet deficient in an essential mineral element, homeostatic mechanisms are brought into play which tend to conserve the element, and make more effective use of the limited amounts which are available. This is particularly true in young animals in which active growth increases basic requirements and imposes additional stress.

In the case of calcium and phosphorus, it has long been recognized that a low level of either of these elements in the diet -- particularly in association with a very high or very low Ca/P ratio and a lack of vitamin D -- may give rise to a condition of rickets in growing animals, with impaired mineralization of the newly formed osteoid matrix (Shohl, A.T., 1939). However, in the early days it was difficult to prepare a synthetic diet which was extremely low in phosphorus because the usual protein source in such diets was skim milk or casein -- both of which are rather high in phosphorus. Day and McCollum (1939) first reported the production

of severe phosphorus deficiency in growing rats restricted to a diet containing 0.017% P in which purified gelatin and edestin were used as source of protein. Despite the presence of 3.8 I.U. vitamin D per gram, these rats developed severe rickets, and there was progressive loss of mineral from the skeleton which ultimately led to death from collapse of the rib cage and asphyxia. An improved phosphorus deficient diet was developed by Coleman et al. (1950) using purified beef blood fibrin as the protein source. With modifications, this is the basic diet used in the following investigations.

A number of recent reviews have discussed the factors involved in calcium homeostasis (Copp, 1957; Howard, 1957; McLean, 1958). These include the balance between absorption and excretion in faeces, urine and sweat, the labile and stable reservoir of mineral in bone (Owen, 1952) and the function of the parathyroid glands (McLean, 1957). These factors also appear to be important in phosphorus homeostasis (Crawford, 1951). Mineral balances, changes in the calcium and phosphorus content of tissues, and in urinary excretion of these elements can be determined by ordinary chemical analysis. However, radiocalcium must be used to determine the subtle changes in the kinetics of calcium movement in and out of bone in the deficient animals. Bauer, Carlsson and Lindquist (1955, 1957) have developed two ingenious methods for estimating the accretion of calcium by bones and teeth, the

exchangeable calcium pool, and the resorption or removal of calcium from these structures. Their method has been used in the following experiments to determine the changes which occur in calcium and phosphorus deficiency in young and adult rats.

METHODS

Animals The animals used were female rats of the Wistar strain. The young rats were weaned at 3½-4 weeks (50-55 g. body weight) and were maintained on the experimental diet for 5 weeks, at which time Ca^{45} was administered and the various kinetic studies were carried out. Mineral balances were followed by analyzing the dietary intake of calcium and phosphorus, and comparing this with the output in urine and feces.

The young adult animals weighed 195-205 grams and were 4 months of age when placed on the experimental diets. They were maintained on these diets for up to 26 weeks. Mineral balances were also carried out in connection with the adult animals. The diet was that described by Coleman et al. (1950) and contained 40 USP units of vitamin D per gram. The control diet assayed 0.40% Ca and 0.45% P; the calcium deficient diet contained 0.037% Ca and 0.45% P; the phosphorus deficient diet contained 0.40% Ca and 0.018% P.

Analytical Methods Plasma calcium was determined by photometric titration with EDTA using a modification of the method of Lehmann (1953). Calcium in tissues and excreta was determined by a

modification (Comar et al., 1951) of the method of Clark and Collip (1925). Inorganic phosphorus was determined in all samples by the Sumner (1944) modification of the method of Fiske and Subbarow. Radiocalcium was determined by the method of Comar et al., (1951) making suitable corrections for self absorption.

The Ca^{45} used had a high specific activity (2 mc./mg.Ca); the dose administered by intraperitoneal injection to each animal contained 10 c. Ca^{45} and only 5 g. Ca.

Kinetic analysis of calcium uptake in the skeleton

In the following experiments, two methods of analysis have been used to study the uptake and retention of calcium in bones, teeth and total skeleton.

Method A. This is a short term method which involves sampling plasma and bones, teeth and carcass at 8, 16 and 24 hours after administration of a dose of Ca^{45} . The method was developed by Bauer, Carlsson and Lindquist (1955), and the assumptions and calculations are described in their paper. They assume that the Ca^{45} is taken up either in a rapidly exchangeable calcium pool in bone ("E"), which reaches equilibrium with the plasma calcium in less than 8 hours; or it is irreversibly deposited in bone by a process which they have described as accretion ("A"). Since the accretion is always greater than the observed net increment of calcium in bone due to growth, they have attributed the difference to resorption ("R"). It should be pointed out that other workers (Marshall, Rowland and Jowsey, 1959) feel that a significant part

of the Ca^{45} accretion so measured may be due to a slow long-term exchange process, particularly in the adult animal.

Method B This method was also developed by Bauer, Carlsson and Lindquist (1957), and is described in detail in their paper. It is based on the assumption that within a few hours after injection, Ca^{45} reaches equilibrium with the calcium in an exchangeable pool ("W") involving plasma, soft tissues and the rapidly exchangeable calcium of the skeleton. From this pool, Ca^{45} is lost by excretion, and by irreversible deposition in the skeleton by accretion ("A"). The same objections have been raised to the precise definition of this term. However, there is general agreement that the figure for accretion is related to the activity of bone formation and mineralization.

Initial clearance of Ca^{45} by bone.

Fredrickson, Honour and Copp (1955) have pointed out that the initial Ca^{45} clearance from plasma by bone probably gives a valid measure of effective bone blood flow. In the method used, Ca^{45} uptake by the bone is determined directly, and this is divided by the integrated plasma concentration of Ca^{45} (% dose/ml.) during the clearance period, determined graphically.

Parathyroid studies

Parathyroidectomy was performed by the Erdheim method of electrocautery described by Greep (1955). The parathyroid extract used was supplied by the E. Lilly Co., and contained 100 USP units per ml.

Cortisone treatment To suppress bone growth, hydrocortisone was administered to young control and phosphorus deficient rats in a dose of 100 mg./kg. by intramuscular injection, and repeated two days later. The animals were killed 24 hours after the second dose. The results of this experiment are now being analyzed.

Tetracycline staining of new bone. Tetracyclines provide an elegant method of staining new bone in vivo, and has been demonstrated by Frost (1960) and others. It is now being used to study actual bone formation in control and deficient animals, using a dose of 5 and 100 mg./kg.

III

EXPERIMENTS AND RESULTS

A. Effects of severe calcium and phosphorus deficiency in young growing rats.

Growth curves for the young animals are shown in Figure 1. The weight gain on the low calcium diet was comparable to that of the controls, as was also true in the young adults. However the young animals ^{which} were restricted to the phosphorus deficient diet grew much more slowly and indeed stopped growing completely after the first two or three weeks. Balance studies carried out during the final week on the experimental diets are shown in Table 1. It should be noted that there is a high net absorption of calcium in both deficient groups; in the calcium deficient animals most of this is retained, while in the phosphorus deficient rats a large part of it is subsequently excreted in the urine. The deficient animals are

practically in balance with respect to calcium and phosphorus; the rats on the control diet show a marked positive retention of both elements.

Changes in femur size are listed in Table 2 and shown diagrammatically in Figure 2 with respect to the phosphorus deficient and control animals only. When compared to the dimensions at the onset of the experiment, the controls show a definite increase in length and in all other dimensions and a three-fourfold increase in the calcium and phosphorus present. The increase in femur length in the phosphorus deficient rats is largely accounted for by the widened rachitic epiphyseal cartilage; all other dimensions are reduced. The shaft is less than half as thick, and there is actually less calcium and phosphorus in the bone than at the onset of the experiment. It is evident that bone growth in these animals is practically nil, and this has been confirmed in some preliminary studies with tetracyclines.

The calcium and phosphorus concentrations in the soft tissues are shown in Table B and Figure 3. The phosphorus concentration in plasma is significantly reduced in the phosphorus deficient group, but normal phosphorus levels are maintained in the other soft tissues. The calcium concentration appears to be slightly higher in the soft tissues of the deficient animals. Values for femur and incisors are shown in Table W, Figure 3. The mineral content of the femur was reduced in both deficient groups while there was no significant change in the mineral content of the incisors. It is evident that the incisors can continue to grow and mineralize

well even in the presence of a severe deficiency of calcium or phosphorus, while the bone suffers. These results confirm the view that the phosphorus level in soft tissues is maintained at the expense of the phosphorus stores in bone.

Figure 4 shows the effects of an interesting experiment on the effects of fasting in phosphorus deficient animals. Within a few hours the plasma calcium falls while the plasma inorganic phosphate rises. These changes are consistent with the view that the phosphorus deficient animal has hypo-functional parathyroid glands, the normal calcium level in plasma being maintained because of the very high absorption rate and the low deposition in bone. An experiment was carried out to estimate bone blood flow in calcium deficient and control rats by measuring initial radiocalcium clearance, as described by Fredrickson et al. (1955). A basic assumption indicated diagrammatically in Figure 5, is that all of the radiocalcium present in the blood flowing through the blood vessels is completely replaced by exchange with non-radioactive calcium in the large exchangeable calcium pool in bone. Support for this assumption is found in the results reported in Table 5 showing that the clearance for the 0-10 minute interval is essentially the same as that in the 0-5 minute interval. The results also indicate that bone blood flow is higher in the phosphorus deficient group in both absolute and relative terms. This may be explained by the much greater vascularity of the scanty bony tissue present in the deficient animals.

The distribution of Ca^{45} 7 days after administration is shown in Table VI. At this time, over half of the injected dose has been lost by urinary excretion in the phosphorus deficient animals; loss from the calcium deficient and control groups during this period is negligible. It is evident that the loss in phosphorus deficiency has been chiefly from bone. Despite this loss, the incisors in these animals show a high retention that exceeds even that in the control group. This confirms the observation that the incisors continue to deposit and retain calcium normally in severe phosphorus deficiency.

The calcium kinetics were investigated in these same groups of animals, and the coefficients as determined by method A (Bauer, Carlsson and Lindquist, 1956) are shown in Table VII and Figure 6; those determined by method B (Bauer, Carlsson and Lindquist, 1957) are shown in Table VIII. The exchangeable calcium in femur is somewhat reduced in both deficient groups, but represents a larger percentage of the total calcium present. Accretion is reduced significantly in the calcium deficient rats, and to an even greater extent in the phosphorus deficient animals. In the latter it is less than resorption, because the bone is actually losing calcium. In contrast, there is only a slight reduction in the accretion of Ca by the incisors of the deficient animals (this may merely reflect the smaller size). The exchangeable calcium in the incisors of the deficient animals is significantly higher when compared to the controls, and may reflect

some increase in the porosity of the dentine. Accretion by the whole carcass, as measured by method B, is one half the value estimated by the first method in the control group and is reduced even further in the deficient animals. These differences may be due to the factors of slow long-term exchange, which will contribute much more to "A" as calculated by the first method than by the second. Indeed, in the phosphorus deficient rats in which practically no new bone growth can be demonstrated in gross or with tetracycline staining, such slow long-term exchange may account for most of the so-called accretion.

B. Effects of diets low in Calcium and Phosphorus in Young Adult Rats.

The growth curves shown in Figure 7 indicate that there is still some growth occurring in these animals, and there is no significant difference between the deficient and the control groups. Balance studies were carried over the entire 26 week period. The low phosphorus animals remained in calcium and phosphorus balance, but did not show any significant retention; the other two groups were in positive balance and showed gains of both elements. The calcium and phosphorus in plasma, femur, incisors and carcass are shown in Table IX. Plasma P was significantly lower in the phosphorus deficient group; the other values are not significantly different, although there is evidence for increased retention of calcium and phosphorus in the control and calcium deficient group.

Coefficients of calcium kinetics determined by methods A and B are shown in Table X. Accretion "A" as determined by

both methods is essentially the same in the control and low phosphorus animals; accretion tends to be higher in the calcium deficient rats. Since the latter have a compensatory hyperparathyroidism, this may reflect the resulting increase in calcium turnover. It is particularly interesting that the values for "A" as determined by both methods are essentially the same in these adult animals.

Parathyroid Effects

The effects of parathyroidectomy and injection of parathyroid extract were investigated in calcium deficient, phosphorus deficient and control rats. The results of balance studies in young adult rats are shown in Table X. Injections of parathyroid hormone increased the net absorption and retention of calcium in the control and phosphorus deficient groups.

C. Parathyroid Effects

Table XI shows the effect of treatment with parathyroid extract on the absorption and retention of calcium and phosphorus by young adult rats fed the various diets. Net calcium absorption is increased by treatment in all three groups, providing evidence that parathyroid hormone promotes calcium absorption from the gut. However, in the low calcium group, increased gut absorption was balanced by increased excretion of calcium in urine, so that the retention was essentially unchanged. In the animals fed the low phosphorus diet, treatment with parathyroid extract resulted in a large increase in the absorption and retention of phosphorus,

and calcium. In contrast to the effect in the other two groups, calcium excretion in urine was reduced.

Because of the striking changes observed in urinary excretion of calcium in animals treated with parathyroid extract, a more detailed experiment was carried out. The changes in urinary calcium produced by parathyroidectomy and treatment with parathyroid extract in young and adult animals fed the various experimental diets are shown in Table XII. The young calcium deficient rats all died following parathyroidectomy, indicating the importance of the functional hyperparathyroidism in compensating for the deficiency in these animals. Treatment with parathyroid extract significantly increased the urinary excretion of calcium in both control groups, in association with the hypercalcemia. However, this treatment actually reduced the calcium excretion in the phosphorus deficient animals - both young and adult. Since this occurred despite an increase in the plasma calcium, it is suggested that this may be due to increased reabsorption of calcium by the renal tubules. Such an effect has been noted by Kleeman (1960) in human subjects.

The distribution of Ca^{45} in the tissues and excreta of parathyroid treated and untreated adult rats is shown in Table XII. In the control group, treatment with the extract has resulted in a very marked increase in the urinary excretion of Ca^{45} but very little effect on the retention in femur and incisors. The opposite effect was observed in the phosphorus deficient group, in which urinary excretion of Ca^{45} was sharply reduced, while bone retention was

increased. In the calcium deficient animals, such treatment increased urinary excretion of Ca^{45} and reduced the amount retained in bone and carcass. The incisors showed remarkably little difference in all groups - both treated and untreated.

The coefficients of calcium kinetics as determined by method B in the parathyroid treated and untreated adult rats are shown in Table XIV. Accretion was higher in both deficient groups than in the control group, and was enhanced by parathyroid treatment. This may have been due to a greater rate of turnover in these animals -- particularly following administration of parathyroid extract.

The effect of parathyroidectomy on the kinetics of calcium uptake in young growing rats is shown in Table XV. This work was reported by Suiker and Copp (1960) at the fall meeting of the American Physiological Society. The reduction in accretion rate in the parathyroidectomized animals was proportional to the reduction in plasma calcium, suggesting that it may be a function of the calcium ion activity. The exchangeable calcium pool was also reduced in the same proportion. This is consistent with the view that the exchangeable calcium pool in bone has the same calcium ion activity and is in equilibrium with the plasma calcium.

Experiments in progress.

One of the critical questions concerning the coefficients of calcium kinetics calculated by the method of Bauer et al. (1956) is the relative contribution of bone formation and slow long term

exchange to the accretion value. In our opinion, the former may be the most important factor in the normal growing animals, but cannot be as important in the phosphorus deficient rats, in which there is no apparent bone growth. To investigate this problem, we have administered cortisone to control and phosphorus deficient animals to inhibit bone growth, and have injected intraperitoneally achromycin (tetracycline hydrochloride) to demonstrate the actual areas of bone formation. Calcium kinetics is being determined by method A (Bauer et al., 1956). The samples from the first run are now being analyzed. A preliminary crude examination of femurs from these animals, sectioned longitudinally, has indicated practically no tetracycline retention in the phosphorus deficient animals, and what appears to be a significant reduction in the tetracycline labelled bone in the cortisone treated controls.

Freeman and McLean (1941) produced phosphorus deficiency in puppies and noted a marked hypercalcemia associated with a low blood phosphate and severe rickets. We have started 2 puppies on the control diet and 4 on the phosphate deficient diet to produce a similar condition, so that we can study severe phosphate deficiency in an animal large enough to investigate quantitatively the changes produced in renal and parathyroid function in absorption by the gut.

DISCUSSION

Growing rats restricted to a diet very low in calcium (0.037%) but adequate in vitamin D and phosphorus were able to

grow almost as well as the controls. They were in positive calcium and phosphorus balance, and the femur appeared only mildly rachitic, with a widened epiphyseal cartilage. The shaft, however, was thin and the mineral content was less than that of the controls, and this reflected the reduced calcium accretion observed. The animal was able to adapt to the low level of dietary calcium by increased efficiency of intestinal absorption, reduction of urinary loss to practically zero, and a significant reduction in calcium accretion by bone. There appeared to be increased functional activity of the parathyroids, as was also observed by Engfeldt (1950), and this may have facilitated the first two processes, as well as maintaining an almost normal plasma calcium level despite the limited intake.

Adaptation to the phosphate deficient diet is much more difficult, for phosphorus is required for the growth of soft tissues. Indeed, even in the terminal stages, the phosphorus concentration in the soft tissues was not reduced. This is apparently accomplished by diverting phosphorus from the skeleton, and accounts for the extreme osteopenia observed. Loss of phosphorus in urine was almost completely suppressed, and bone growth and mineralization had practically stopped. However, with very low levels of phosphate in the gut, calcium absorption was high. Since it was not deposited in bone, this excess calcium was eliminated in the urine, and calcium excretion was increased up to 20x as compared to the controls. There was considerable evidence that the parathyroids were hypofunctional in these animals. This may facilitate the conservations of phosphorus. The factors involved are shown in Figure 5.

One of the problems is to account for the calcium accretion in these phosphorus deficient animals in which bone growth has stopped. It seems probable that a small but significant component of the accretion value in the normal animals may be due to a very slow exchange process; in the phosphorus deficient animals this may account for most of the accretion observed. Some of the implications of the parathyroid studies are discussed in the previous section.

SUMMARY

When the dietary intake of calcium or phosphorus was severely curtailed, a number of changes occurred which tended to conserve the element and reduce its utilization. In calcium deficiency, loss in urine was suppressed, efficiency of intestinal absorption was increased, and there was a reduction in calcium accretion and bone growth. The parathyroids appeared to be hyperfunctional, and a normal blood calcium level was maintained.

In severe phosphorus deficiency, urinary excretion of this critical element was almost completely suppressed, and it was diverted from the skeleton to the soft tissues, with consequent loss of mineral by the former. However, the incisors continued to grow and calcify almost normally. Calcium accretion by bone was sharply reduced, and there appeared to be essentially no new bone growth. It is evident that, in this condition, the needs of the soft tissues take precedence over those of the skeleton.

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Growth Curves of Young

Female Wistar Rats.

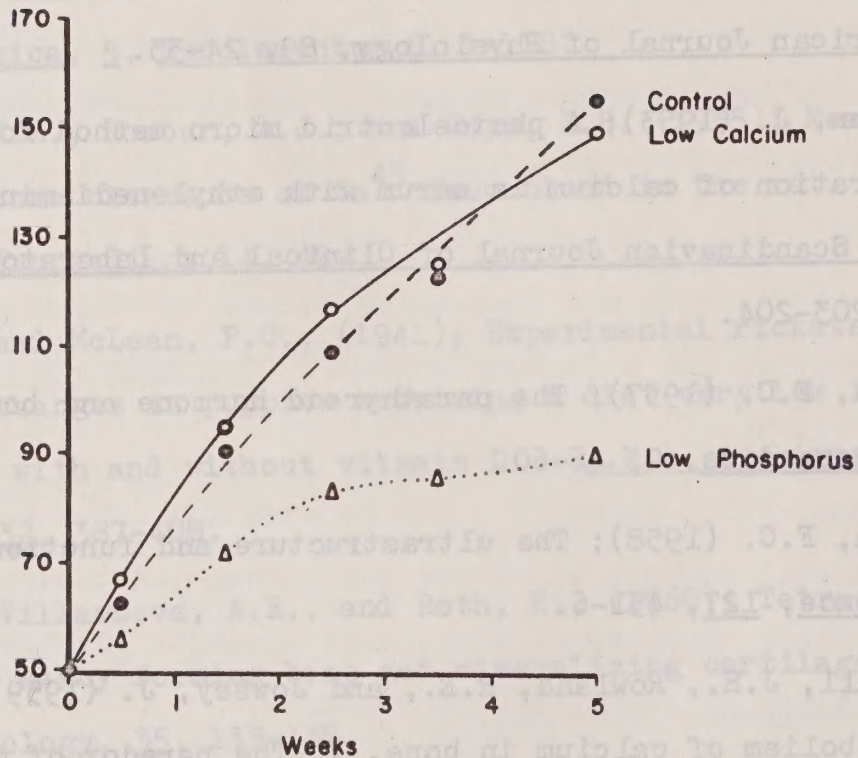
Weight Gain
gms.

Figure 1. Growth curves of young rats restricted from the time of weaning to low calcium, low phosphorus or control diets fed ad libitum.

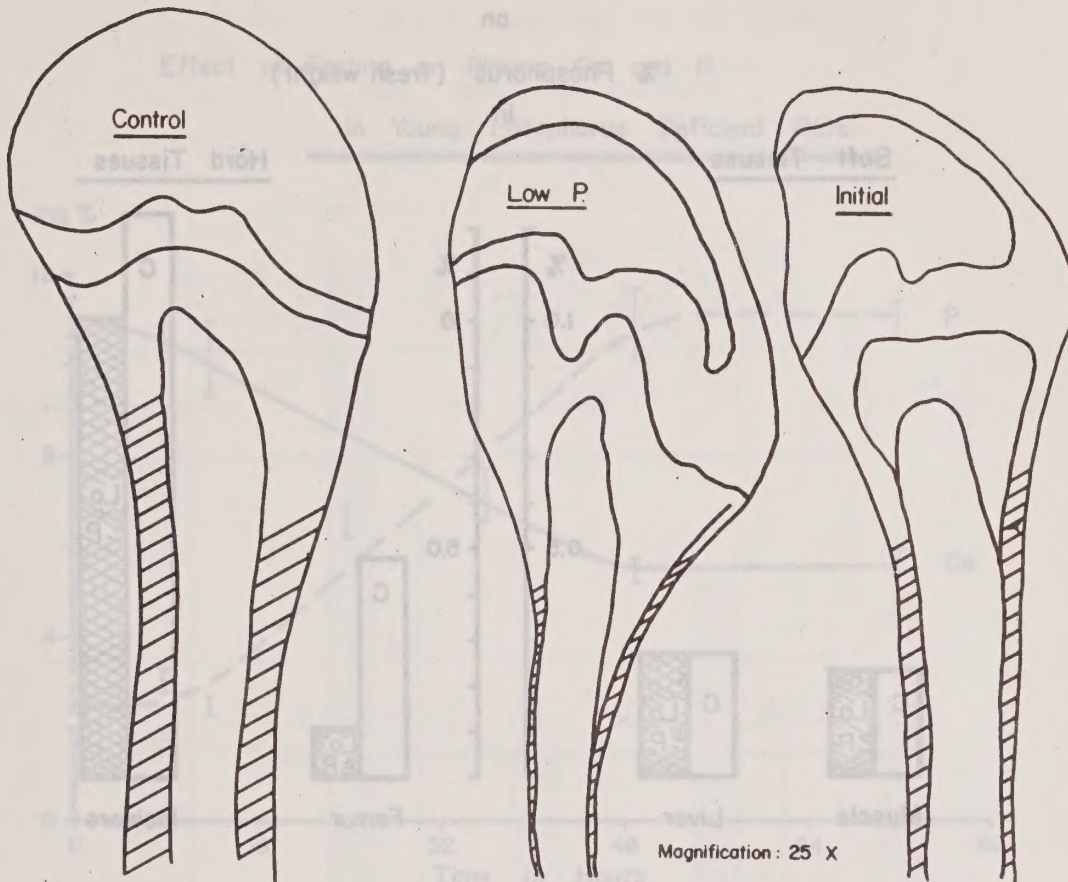


Figure 2. Outline of longitudinal sections of the femurs from
a) rat fed the control diet for 5 weeks from weaning.
b) rat fed the low phosphorus diet for 5 weeks from weaning.
c) 3 week old rat just weaned - at the onset of the experiment

Effect of Severe Phosphorus Deficiency

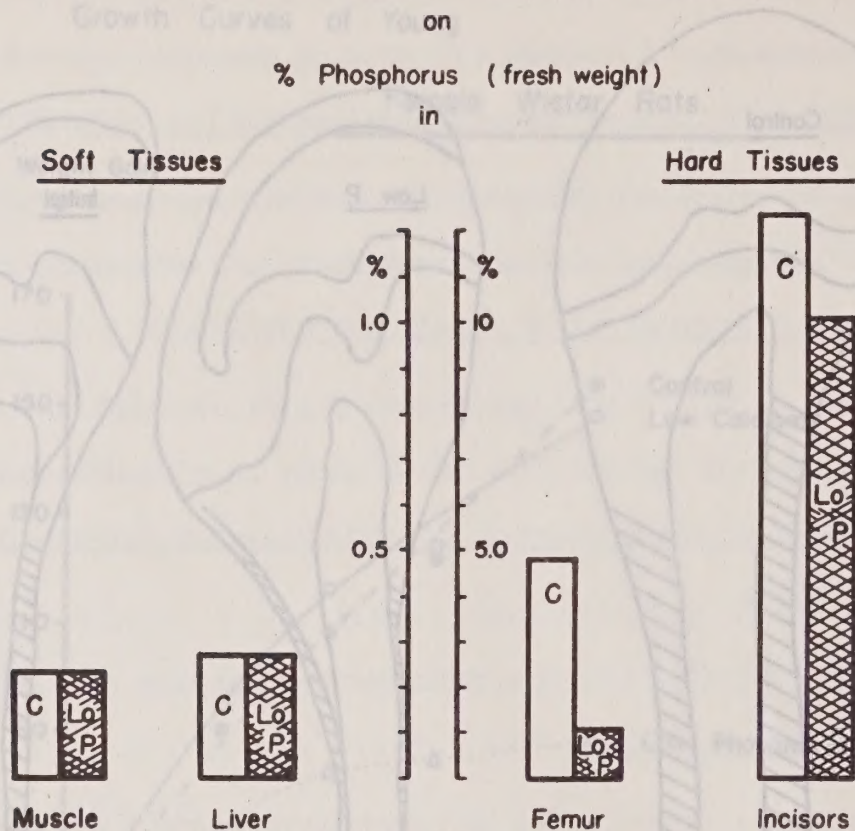


Figure 3. Effect of severe phosphorus deficiency on the percent of phosphorus (fresh weight) in muscle, liver, femur and incisors.

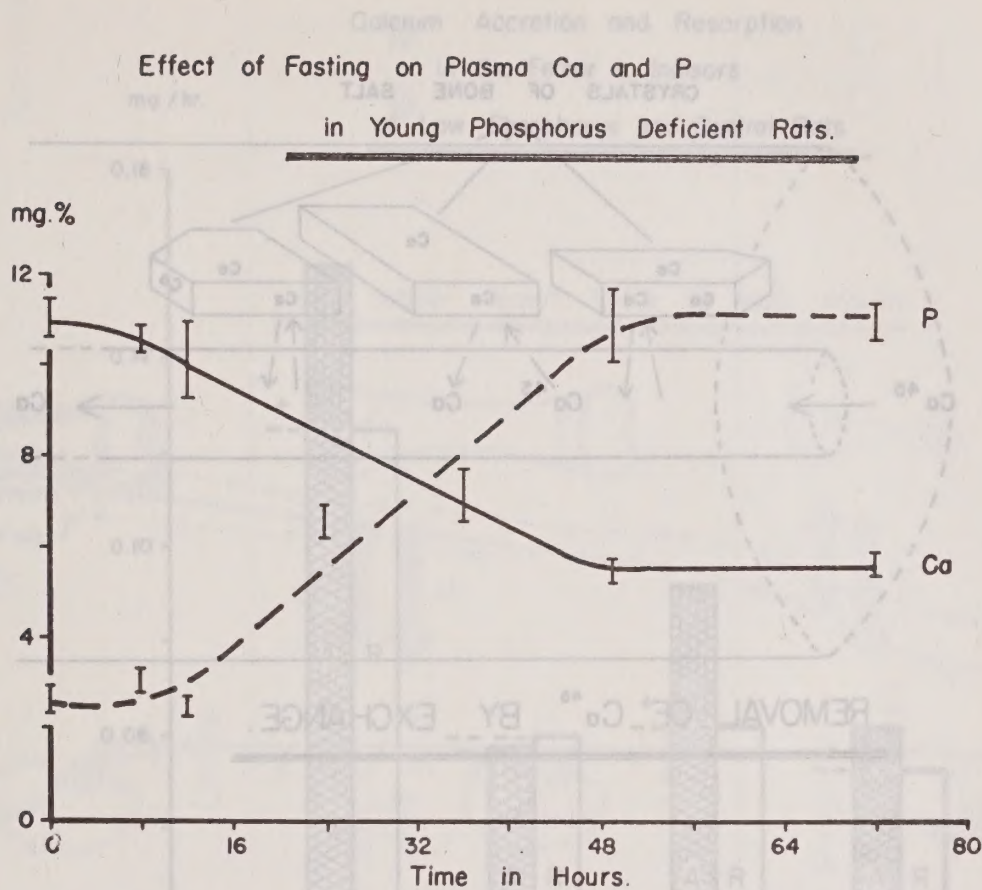


Figure 4. Effect of fasting on the plasma calcium and phosphorus level in young phosphorus deficient rats.

Figure 5. Balance between calcium accretion and resorption in the femur and incisor of young phosphorus deficient and control young rats.

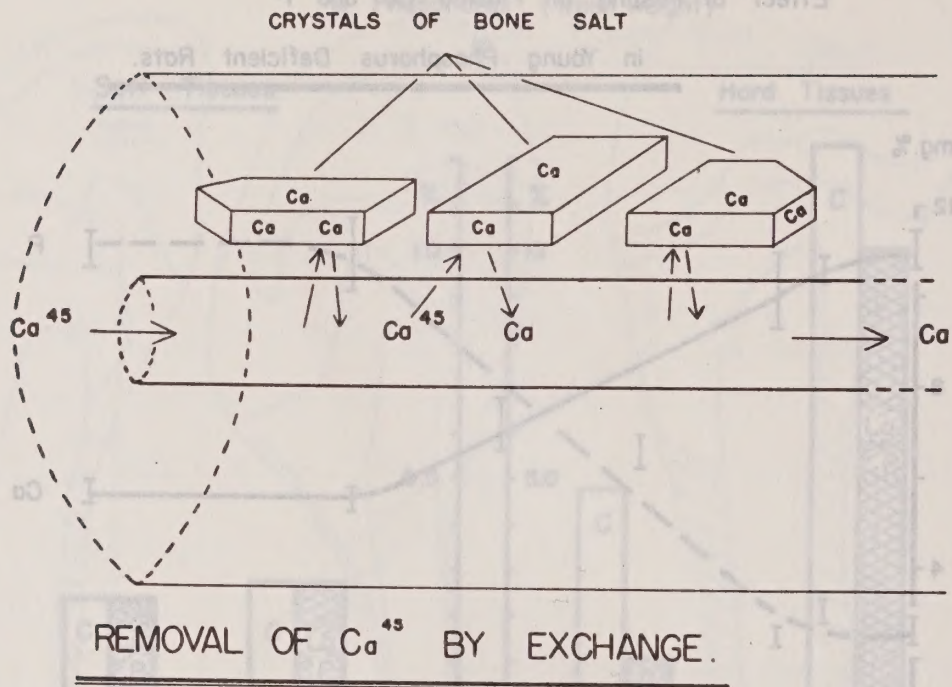


Figure 3. Effect of severe phosphorus deficiency on the percent of phosphorus (fresh weight) in muscle, liver, tamar and incisors.

Figure 5. Diagram showing the proposed mechanism for the initial and complete clearance of Ca^{45} from blood by exchange with the large pool of non-radioactive calcium in bone.

Calcium Accretion and Resorption
in the Femur & Incisors

of Low Phosphorus and Control Rats

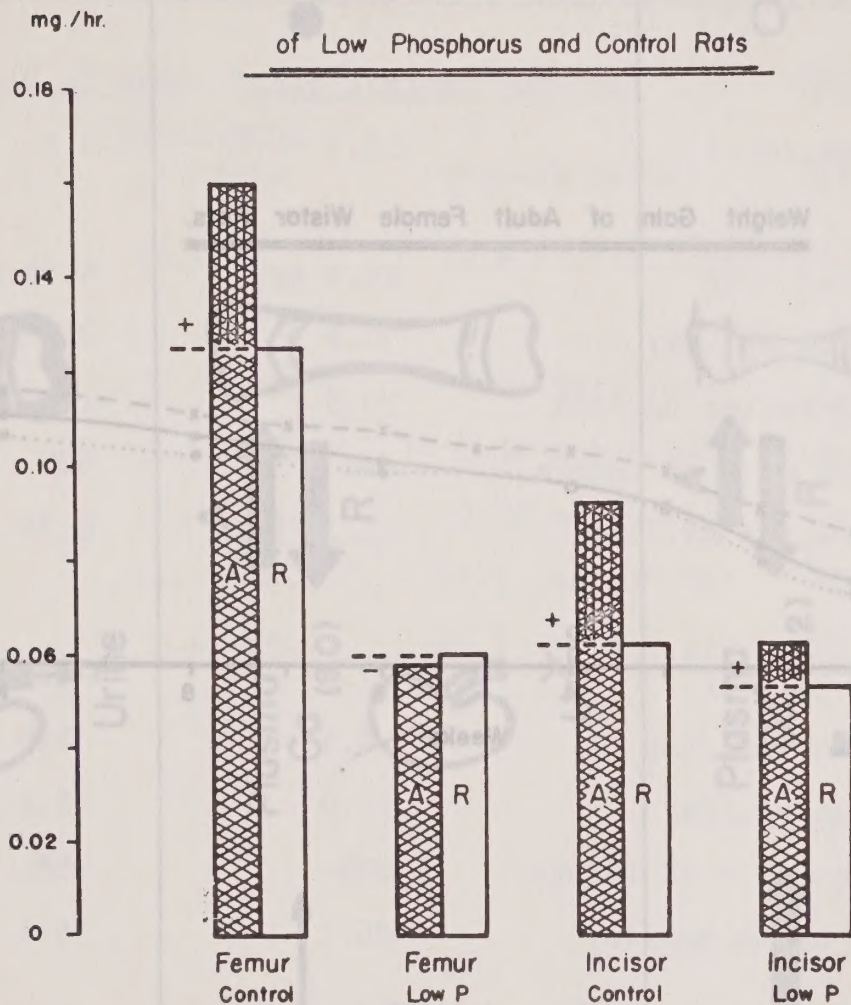


Figure 6. Balance between calcium accretion and resorption in the femurs and incisors of phosphorus deficient and control young rats.

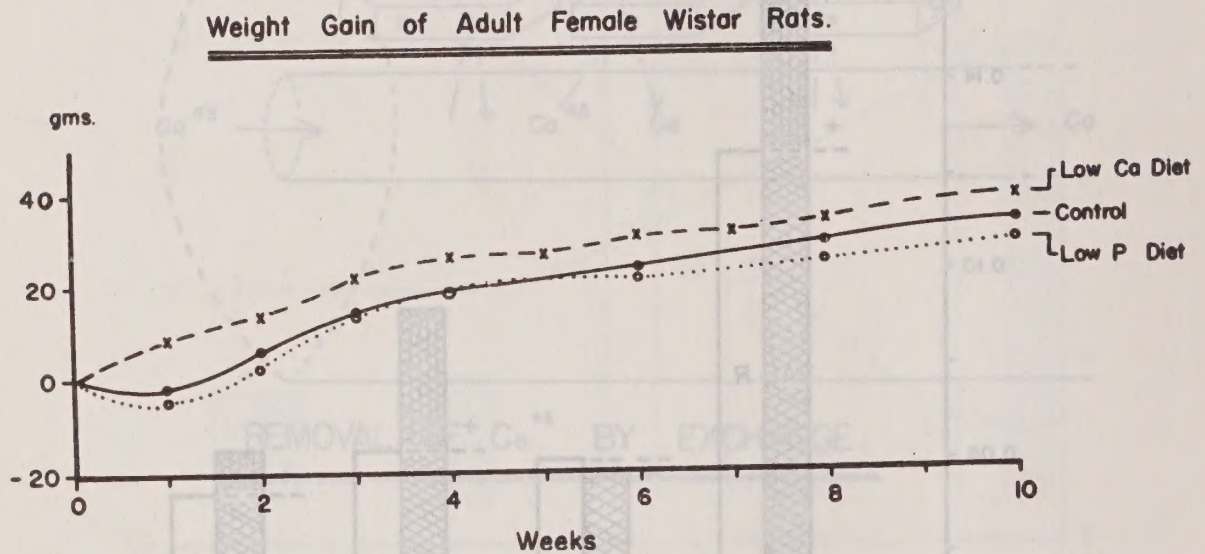


Figure 7. Growth curves of young adult rats restricted to the low phosphorus, low calcium and control diets.

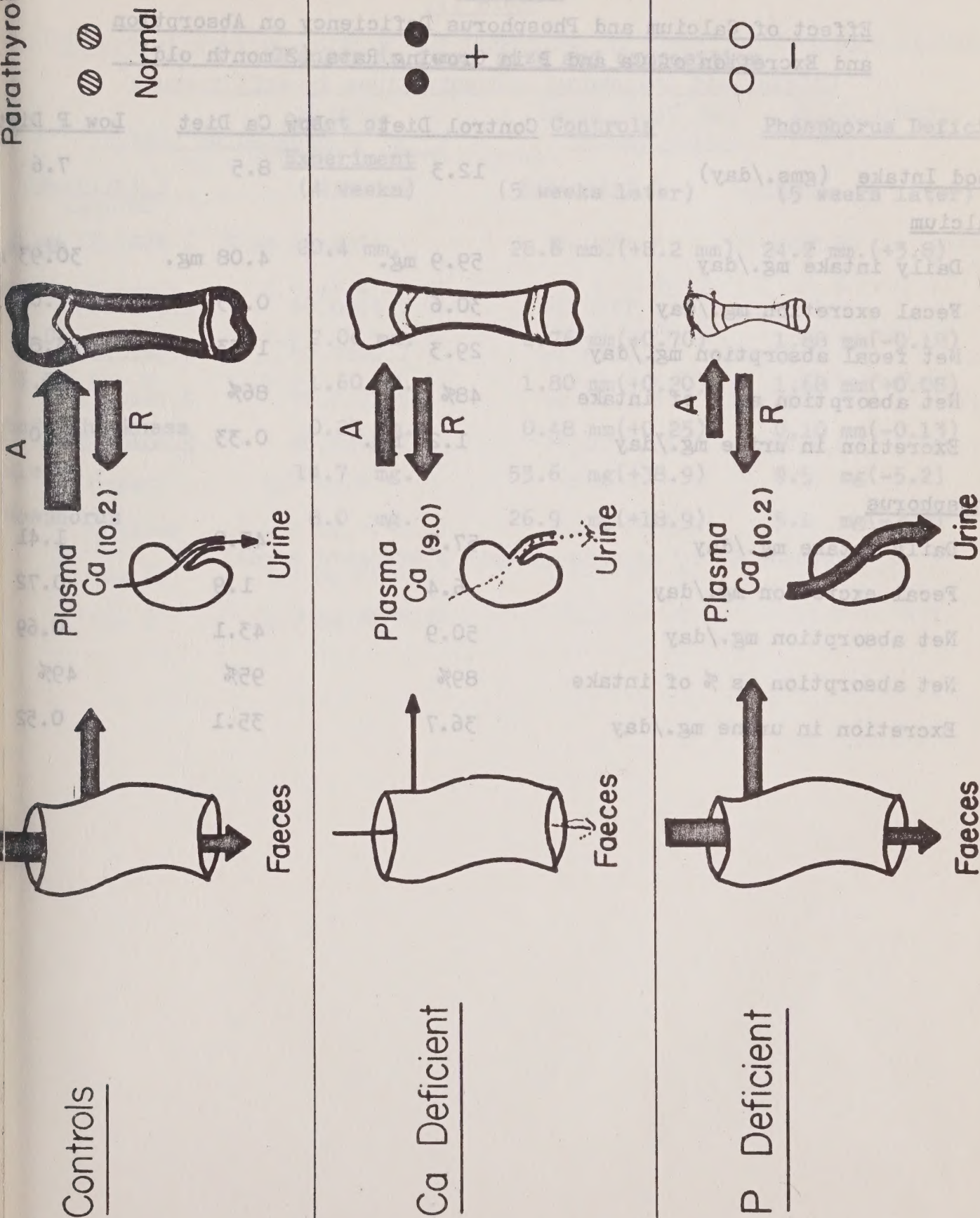


Figure 3. Factors involved in the adaptation of growing rats to dietary deficiency of calcium or phosphorus.

Table I

Effect of Calcium and Phosphorus Deficiency on Absorption and Excretion of Ca and P in Growing Rats (2 month old)

	<u>Control Diet</u>	<u>Low Ca Diet</u>	<u>Low P Diet</u>
<u>Food Intake</u> (gms./day)	12.3	8.5	7.6
<u>Calcium</u>			
Daily intake mg./day	59.9 mg.	4.08 mg.	30.93
Fecal excretion mg./day	30.6	0.55	7.08
Net fecal absorption mg./day	29.3	1.53	23.85
Net absorption as % of intake	48%	86%	77%
Excretion in urine mg./day	1.21 mg.	0.33	19.0
<u>Phosphorus</u>			
Daily intake mg./day	57.3	45.0	1.41
Fecal excretion mg./day	6.4	1.9	0.72
Net absorption mg./day	50.9	43.1	0.69
Net absorption as % of intake	89%	95%	49%
Excretion in urine mg./day	36.7	35.1	0.52

Table IIChanges in femur size and composition

	<u>Onset of</u> <u>Experiment</u> (4 weeks)	<u>Controls</u> (5 weeks later)	<u>Phosphorus Deficient</u> (5 weeks later)
Length	20.4 mm.	28.6 mm.(+8.2 mm)	24.2 mm.(+3.8)
Shaft -			
O.D.	2.06 mm.	2.76 mm(+0.70)	1.88 mm(-0.18)
I.D.	1.60 mm.	1.80 mm(+0.20)	1.68 mm(+0.08)
Shaft thickness	0.23 mm.	0.48 mm(+0.25)	0.10 mm(-0.13)
Calcium	14.7 mg.	53.6 mg(+38.9)	9.5 mg(-5.2)
Phosphorus	8.0 mg.	26.9 mg(+18.9)	5.1 mg(-2.9)

Table III

Calcium and phosphorus concentrations in soft tissues*

	<u>Calcium (mg.%)</u>		<u>Phosphorus (gms.%)</u>	
	<u>Control</u>	<u>P deficient</u>	<u>Control</u>	<u>P deficient</u>
Plasma (mg.%)	9.91±0.15	9.43±0.34	7.24±0.21 mg.%	2.86±0.33 mg.%
Muscle	5.64±0.44	6.77±0.25	2.22±0.04	2.25±0.09
Skin	8.91±0.41	10.05±0.36	1.00±0.03	0.94±0.02
Liver	5.81±0.42	8.51±0.58	2.72±0.87	2.74±0.12
Kidney	18.6 ±1.08	18.4 ±0.75	2.77±0.05	2.80±0.07

* averages of 10 or more animals ± standard error

Table IVEffect of diet on calcium and phosphorus of femur and incisors (young rats

	<u>Plasma</u> <u>mg.% acid sol.P</u>	<u>Total Femur mg.</u>	<u>Total Incisor mg.</u>
<u>CALCIUM</u>			
Onset	10.4 mg.%	14.7	22.3
Control	9.9 mg.%(-0.5)	53.6 (+38.9)	49.5 (+27.2)
Low Ca	9.5 (-0.9)	19.9 (+ 5.2)	44.1 (+21.8)
Low P	11.8 (+1.4)	9.5 (-5.2)	38.6 (+16.3)
<u>PHOSPHORUS</u>			
Onset	8.0 mg.%	8.0	12.7
Control	6.5 mg.%(-1.7)	26.9 (+18.9)	28.1 (+15.4)
Low Ca	7.1 mg.%(-1.1)	10.4 (+2.4)	24.2 (+11.5)
Low P	2.3 mg.%(-5.9)	5.1 (-2.9)	21.0 (+ 8.3)

Table V

Initial Ca^{45} clearance from plasma by femur (estimated effective bone blood flow)

	<u>Femur</u>			
	<u>ml./min.</u>	<u>ml.min./sq.</u>		<u>"Skeletal Blood Flow"</u>
		<u>meter¹</u>		<u>estimated as % resting</u>
				<u>cardiac output²</u>
<u>Control Group</u>				
0-5 minutes	0.0515	2.04		4.3
0-10 minutes	0.0511	2.03		4.3
<u>Phosphorus deficient</u>				
0-5 minutes	0.147	7.53		15.9
0-10 minutes	0.145	7.32		15.5

1. Surface area calculated according to the formula of Lee (1929)
2. Based on the assumption that total skeletal uptake = 25 x femur uptake, and a cardiac index of 2.15 l./min./sq.m. for the rat as given by Hoelscher (1954).

Table VI

Young female Wistar rats : % dose of Ca⁴⁵ 7 days after I.P. injection

	<u>Low Calcium</u>	<u>Low Phosphorus</u>	<u>Control</u>
Serum Sp. Activity	0.17 ± 0.01	0.04 ± 0.01	0.04 ± 0.003
Femur	4.45 ± 0.29	1.64 ± 0.07	4.11 ± 0.03
Incisors	4.33 ± 0.21	4.02 ± 0.25	3.21 ± 0.07
Total Carcass	99.51 ± 0.50	46.34 ± 0.70	99.02 ± 1.23
Gut	0.31 ± 0.02	0.22 ± 0.02	0.24 ± 0.03
Faeces	0.45 ± 0.13	1.90 ± 0.40	2.31 ± 0.14
Urine	0.23 ± 0.03	52.36 ± 2.62	0.43 ± 0.04

Table VIICalcium kinetics - young rats (Method A)

	Accretion (A) <u>mg./hr.</u>	Resorption (R) (5-6 weeks) <u>mg./hr.</u>	Exchangeable Bone Calcium (E) <u>mg.</u>	<u>% total</u>
<u>FEMUR</u>				
Controls	0.161	0.125	2.67	5.0
Low Ca	0.095	0.090	1.49	7.5
Low P	0.056	0.058	1.23	13.0
<u>INCISORS</u>				
Controls	0.092	0.062	0.17	0.34
Low Ca	0.073	0.047	0.48	1.1
Low P	0.062	0.050	0.97	2.5
<u>* CARCASS (- gut)</u>				
Controls	2.93	2.27	53.3	3.8
Low Ca	2.10	2.00	30	6.4
Low P	(1.14 - 1.51)	1.57	33	8.5

* (includes soft tissue)

Table VIII Method (B)

* Calcium kinetics in the young animals (Method B)

Values for Total Carcass					$\frac{\% \text{ Dose}}{\text{Carcass}}$
A	E	% E	U	$T\frac{1}{2}$ Hrs.	
0.723	148	32	0.0366	285	96.54
0.423	65	22	0.416	53	55.66
1.58	132	12	0.793	55	95.63

* (includes soft tissues)

mg		mg		mg	
Weight	Livers	Weight	Livers	Weight	Livers
503	2.08	503	2.08	503	2.08

Chemical content of young animals after 24 hours on the diet

Table IX

Chemical content of adult Wistar rats after 26 weeks on the diet

Weight #	Plasma mg%		Femur mg		Incisors mg		Carcass mg	
	Ca	P	Ca	P	Ca	P	Ca	P
Onset	9.83	5.68	85.88	40.54	71.67	40.39	2076	1237
	0.83	0.70	3.27	1.46	1.61	1.11	59	41
Control I	10.20	2.34	103.4	49.98	93.60	49.88	2500	1450
	0.30	0.03	4.4	1.91	1.29	1.91	14	54
Low Ca	9.00	2.97	93.20	44.74	91.20	51.30	2300	1410
	0.58	0.27	2.47	1.69	1.82	1.19	63	44
Low P	10.25	1.49	84.88	41.50	87.76	48.10	2150	1280
	0.25	0.23	3.45	1.90	2.33	1.30	54	27

* Calcium kinetics in the adult animals (Method B)

Group	Accretion (A)	Resorption (B)	Calcium (C)
Onset	0.095	0.090	0.012
Control I	0.062	0.050	0.012
Low Ca	0.095	0.090	0.012
Low P	0.095	0.090	0.012

Table X

Calcium kinetics in the adult female Wistar rat.

<u>Total Carcass</u>	<u>A</u> <u>mg/hr.</u>	<u>E mg.</u>	<u>%E</u>	<u>% Excret.</u>	<u>U</u>	<u>T½ days</u>	<u>K</u>	<u>S_b</u>	<u>S_m</u>
Low calcium	1.02	79	3.65	5.44	0.64	58	0.012	0.087	84.9
Low phosphorus	0.86	96	4.61	37.45	0.556	47	0.015	0.049	67.4
Control	0.85	140	5.87	27.55	0.377	75	0.009	0.081	68.8

Values obtained using Bauer short term equations (Method A)

	<u>mg/hr</u>	<u>E mg</u>	<u>% E</u>
Low calcium	1.02	82.7	3.83
Low phosphorus	0.90	42.8	2.05
Control	0.81	172.0	7.22

Table XI

Experiment 14

Balance study of rats (young adult rats approx. 20 weeks of age) on a low calcium, low phosphorus and control diet with and without P.T.H.

	Faeces		Urine		Net Absorbed		Retention	
	Ca	P	Ca	P	Ca	P	Ca	P
Low Ca no P.T.H.	46%	9%	12%	52%	54%	91%	42%	39%
with P.T.H.	28%	5%	34%	37%	72%	95%	38%	68%
Low P no P.T.H.	63%	100%	29%	13%	37%	0%	8%	13%
with P.T.H.	27%	74%	8%	190%	73%	26%	65%	164%
Control no P.T.H.	85%	38%	1%	42%	15%	62%	14%	20%
with P.T.H.	59%	26%	6%	35%	41%	74%	35%	39%

Values are expressed as % of ingested chemical excreted or retained over a three day period. Animals were on the diet 8 weeks at which time the animals received 50 units of P.T.H. at 8 a.m. and 5 p.m. each day for 3 days.

The low calcium diet contained (0.034% Ca and 0.416% P); low P (0.416% Ca and 0.021% P) control (0.403% Ca 0.507% P)

Table XIII

Effect of parathyroidectomy and parathyroid hormone in control, Ca and P
deficient rats

	<u>Young Rats</u>		<u>Adult Rats</u>	
	<u>Excretion of Ca[*]</u> <u>mg./day</u>	<u>% of ingested</u> <u>calcium</u>	<u>Excretion of</u> <u>Ca mg./day</u>	<u>% of</u> <u>ingested</u> <u>calcium</u>
<u>Control Diet</u>				
a) intact untreated	1.31	2.1%	2.15	4.7%
b) parathyroidectomized	0.54	2.9%	- (not done)	-
c) intact treated with parathyroid extract	3.61	6.9%	3.49	16.1%
<u>Low Phosphorus Diet</u>				
a) intact untreated	19.0	61.6%	12.3	48.0%
b) parathyroidectomized	12.4	41.4	- (not done)	-
c) intact treated with** parathyroid extract	6.29	28.4	3.57	17.8%
<u>Low Calcium Diet</u>				
a) intact, untreated	0.33	10.6%	0.29	13.8%
b) parathyroidectomized	-(all animals died)	-	- (not done)	-
c) intact - treated with parathyroid extract**	0.67	17.7%	4.74	23.0%

* Daily excretion based on a 6 day collection period.

** Parathyroid extract in dose of 200 USP units/kg. given subcutaneously twice daily for the six day period. Hypercalcemic response obtained throughout.

Table XIII

Adult female Wistar rats. % dose Ca^{45} present in tissues 6 days after I.P. injection

	Low Calcium				Low Phosphorus				Control	
	P.T.H.		P.T.H.		P.T.H.		P.T.H.		P.T.H.	
	untreated	treated	untreated	treated	untreated	treated	untreated	treated	untreated	treated
Serum sp activity	0.412	0.168	0.095	0.100	0.251	0.117	0.251	0.117	0.117	0.117
Femur	3.88±0.02	2.76±0.16	2.44±0.38	3.52±0.04	2.08±0.18	1.96±0.11	2.08±0.18	1.96±0.11	1.96±0.11	1.96±0.11
Incisors	8.76±0.21	9.30±0.59	4.83±0.48	4.36±0.76	9.98±0.25	8.88±0.51	9.98±0.25	8.88±0.51	8.88±0.51	8.88±0.51
Total carcass	93.7± 2.5	76.4 ±1.8	64.8 ±2.6	92.3± 1.9	65.0 ±0.4	54.1 ±1.8	65.0 ±0.4	54.1 ±1.8	54.1 ±1.8	54.1 ±1.8
Gut	0.49±0.07	0.55±0.30	0.58±0.29	0.51± 0.21	1.08±0.07	0.57±0.33	1.08±0.07	0.57±0.33	0.57±0.33	0.57±0.33
Total faeces	2.83	5.76	2.85	1.80	30.88	24.29	30.88	24.29	24.29	24.29
Total urine	0.59	16.25	30.16	3.82	1.96	17.94	1.96	17.94	17.94	17.94

Table XIV

Calcium kinetics in the adult Wistar rat which has been on a diet for 26 weeks

The effect of P.T.H.

	<u>A</u>	<u>E mg</u>	<u>T½ hrs</u>	<u>U</u>	<u>K</u>	<u>S_b</u>	<u>S_m</u>
<u>Low Phosphorus Diet</u>							
+ P.T.H.	0.610	50.8	44	0.193	0.016	0.168	114.0
- P.T.H. (untreated)	0.436	44.9	68	0.023	0.010	0.412	177.8
<u>Low Calcium Diet</u>							
+ P.T.H.	1.12	79.1	46	0.073	0.015	0.100	77.1
- P.T.H. (untreated)	0.707	59.8	38	0.381	0.018	0.095	86.7
<u>Control Diet</u>							
+ P.T.H.	0.322	32.2	39	0.251	0.018	0.117	168.3
- P.T.H. (untreated)	0.390	38.1	44	0.212	0.016	0.251	150.1

Table XV

Kinetics of calcium uptake in young normal and
parathyroidectomized rats (Method A)

<u>Femur</u>	<u>Control</u> <u>mg./hr.</u>	<u>Parathyroidectomized</u> <u>mg/hr.</u>
Accretion rate (A)	0.118 (0.124 - 0.111)	0.068 (0.073 - 0.062)
<u>A</u> mg. Ca/ml. plasma	1.21	1.13
% Exchangeable (E)	5.0 (4.76 - 5.23)	3.05 (3.23 - 2.86)
<u>E-mg</u> mg. Ca.ml. plasma	12.0	12.0

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH
NATIONAL RESEARCH COUNCIL OF CANADA

Grant No. DA-75

Grantees: C. R. Castaldi and K. A. McMurchy

- Purpose:
1. C. R. Castaldi reported in 1960 on the first phase of this study, namely, "To tabulate the extent and distribution of enamel faults in a group of children with cerebral palsy and in a control group".
 2. The present report is on phase two, carried out by K. A. McMurchy, which involved "a microscopic study of ground sections of primary teeth obtained from children with known cerebral damage, in order to obtain more accurate information regarding the time of cerebral damage".

Method

Several ground sections were made from each tooth selected for study. The sections were dehydrated in alcohols and mounted in permount. Drawings were made of the sections and all accentuated incremental lines in enamel and dentin were recorded. Neighboring sections were checked to eliminate the recording of artifacts. Standard drawings were made showing the normal position of the neonatal line on each type of primary tooth and these were used as a guide in deciding which accentuated line was the neonatal line. In cases where birth information was available, it too was used to decide which line was the neonatal disturbance.

Results

The following chart shows the occurrence of accentuated incremental lines as recorded from observations of the enamel and dentin of primary teeth of 58 patients.

Patient	Months after Conception																		Physical Condition
	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	
75-1	-	-	-	-	X	-	-	-	-	-	-	-	-	-	-	-	-	-	Normal
75-34	-	-	-	-	X	-	-	-	-	-	-	-	-	-	-	-	-	-	Normal
75-35	-	-	-	-	X	-	-	-	-	-	-	-	-	-	-	-	-	-	Normal
75-46	-	-	-	-	X	-	-	-	-	-	-	-	-	-	-	-	-	-	Normal
75-49	-	-	-	-	X	0	-	-	-	-	-	-	-	-	-	-	-	-	Normal
75-7	-	-	-	-	X	-	-	-	-	-	-	-	-	-	-	-	-	-	Normal
75-5	-	-	-	-	X	-	-	-	-	-	-	-	-	-	-	-	-	-	Normal
75-38	-	-	-	-	X	-	-	-	-	-	-	-	0	-	-	-	-	-	Normal
75-51	-	-	-	-	X	-	-	-	0	-	-	-	-	-	-	-	-	-	Normal
75-53	-	-	-	X	-	0	-	-	-	-	0	-	-	-	-	-	-	0	Normal
75-6	-	-	-	X	-	-	-	-	-	-	-	-	0	-	-	-	-	-	Normal
75-14	-	-	-	X	-	-	-	-	-	-	-	-	0	-	-	0	-	-	Normal
75-24	-	-	-	0	X	-	-	-	-	-	-	-	0	-	-	0	-	-	Normal
75-26	-	-	-	X	-	-	-	-	-	-	-	-	0	-	-	0	-	-	Normal
75-40	-	-	-	X	-	-	-	-	-	-	-	-	-	-	-	-	-	0	Normal
75-50	-	-	-	X	-	-	-	-	-	-	-	-	0	-	-	0	-	-	Normal
75-56	-	-	-	X	-	-	-	-	-	-	-	-	0	-	-	-	-	-	Normal
75-58	-	-	-	X	0	-	-	-	-	-	-	-	0	-	-	-	-	-	Normal
75-59	-	-	-	X	-	-	-	-	-	-	-	-	0	-	-	-	-	-	Normal
75-16	-	-	-	X	0	-	-	-	0	-	-	-	-	-	-	-	-	-	Spastic Paraplegia
75-19	-	-	-	X	-	-	-	-	-	-	-	-	-	-	-	-	-	-	Spastic Paraplegia
75-25	-	-	-	-	-	-	-	-	-	0	-	0	-	-	-	-	-	-	Spastic Paraplegia
75-30)	-	-	0	-	X	-	-	-	-	-	-	-	-	-	-	-	-	-	Spastic Paraplegia
75-41)	-	-	0	-	X	-	-	-	-	-	-	-	-	-	-	-	-	-	Spastic Paraplegia
75-2	-	-	0	-	X	-	-	-	-	-	-	-	-	-	-	-	-	-	Left Hemiplegia
75-8	-	-	-	-	X	-	-	-	-	-	-	-	-	-	0	-	-	-	Left Hemiplegia
75-11	-	-	0	-	0	-	X	-	-	-	-	-	-	-	-	-	-	-	Left Hemiplegia
75-20	-	-	-	-	X	-	-	-	-	-	-	-	-	-	-	-	-	-	Spastic Hemiplegia
75-22	-	-	-	-	X	-	-	-	-	-	-	-	-	-	-	-	-	-	Spastic Hemiplegia
75-55	-	-	-	-	X	-	-	-	-	-	-	-	-	-	-	-	-	-	Spastic Hemiplegia
75-31	-	-	-	-	X	-	-	-	-	-	-	-	-	-	-	-	-	-	Spastic Hemiplegia
75-37	-	-	-	-	X	0	-	-	-	-	-	-	-	-	-	-	-	-	Spastic Hemiplegia
75-47	-	-	-	-	X	-	-	-	-	0	-	-	0	-	-	-	-	-	Spastic Hemiplegia
75-4	-																		

Conclusions

1. Most of the normal children show neonatal lines around the ninth month.

If the child was premature it still showed a disturbance at approximately the ninth month which suggested a physiological factor. This same pattern was exhibited in most of the cerebral palsy cases as well.

However, these nine month lines could just as well be disturbances associated with the prematurity or unknown factors.

2. The normal children showed many disturbances of unknown etiology between the 5th. and 9th. month after birth.

3. Prematurity as shown by a shift to the left of the Xs in Figure 1, was commonest in the spastic paraplegia and diplegia group.

4. Seven disturbances of unknown etiology were recorded in prenatal enamel: These occurred in cases in which the physical condition was as follows:-

- 1 Normal - term birth
- 1 Spastic paraplegia - premature birth
- 2 Left hemiplegias - one premature, one term birth.
- 1 Athetoid quadriplegia
- 2 Ataxias - both term births

5. Three yellowish pigmented lines in dentin were noted: These occurred in cases in which the physical condition was as follows:-

- 1 Athetoid quadriplegia - pigmented at 7 months after birth.
- 1 Quadriplegia - at 3 months after birth.
- 1 Aspartic quadriplegia - at 2 months after birth (history of encephalitis at 7 weeks)

Discussion

1. The period of development of the patient covered by enamel examination was limited in most of our cases to the developmental period of one tooth. An effort is being made to collect a primary molar in those cases in which only incisors have been examined to date. If a primary central and a primary second molar could be obtained from each child it would provide an examination period of from 4 months in utero to one year after birth.

2. More accurate estimates of the time between disturbances and the length of disturbances is to be attempted by actual counts of the rod segments between disturbances or involved in disturbances.
3. More cases are needed in all diagnostic groups before a statistical analysis is attempted.

Respectfully submitted,

K. A. McMurchy

K. A. McMurchy, D.D.S.

Members of the Associate Committee on Dental
Research and its Executive, with their terms of
appointments

Term to Expire

- | | | |
|------|---|----------------|
| * 1. | Dr. K. J. Paynter, <u>Chairman</u>
Division of Dental Research,
University of Toronto,
Toronto, Ont. | 31 March, 1962 |
| 2. | Dr. E. W. R. Steacie, <u>ex officio</u> ,
President,
National Research Council,
Ottawa 2, Ont. | - - - |
| 3. | Dr. R. F. Farquharson, <u>ex officio</u> ,
Vice-President (Medical),
National Research Council,
Ottawa 2, Ont. | - - - |
| 4. | Dr. D. L. Thomson, <u>ex officio</u> ,
Vice-Principal,
McGill University,
Montreal, P. Q. | - - - |

Representing the Dental Profession

- | | | |
|------|--|----------------|
| 5. | Dr. C. Castaldi,
Faculty of Dentistry,
University of Alberta,
Edmonton, Alta. | 31 March, 1963 |
| 6. | Dr. L. E. Francis,
Department of Clinical Dentistry,
McGill University,
Montreal, P. Q. | 31 March, 1962 |
| * 7. | Dr. I. Kleinberg,
Department of Biochemistry,
University of Manitoba,
Winnipeg, Man. | 31 March, 1962 |

* Member of the Executive

* Member of the Executive

Term to Expire

8. Dr. J.W. Neilson,
Dean, Faculty of Dentistry,
University of Manitoba,
Winnipeg, Man. 31 March, 1962

9. Dr. G. Nikiforuk,
Division of Dental Research,
University of Toronto,
Toronto, Ont. 31 March, 1963

Representing the Royal Canadian Dental Corps,
Department of National Defence

10. Brig. K.M. Baird, ex officio,
Director General of Dental Service,
Royal Canadian Dental Corps,
Department of National Defence,
Ottawa, Ont. - - -

Representing the Division of Dental Health,
Department of National Health and Welfare

11. Dr. H.K. Brown, ex officio,
Chief, Dental Health Division,
Department of National Health and Welfare,
Ottawa, Ont. - - -

Representing Dental Research,
Department of Veterans Affairs

12. Dr. L.A. Kilburn, ex officio,
Director of Dental Research,
Department of Veterans Affairs,
Ottawa, Ont. - - -

Representing the Basic and Medical Sciences

13. Dr. L.F. Belanger,
Department of Histology,
University of Ottawa,
Ottawa, Ont. 31 March, 1962

* 14. Dr. A. D'Iorio,
Department of Physiology,
University of Montreal,
Montreal, P.Q. 31 March, 1961

* Member of the Executive

Term to Expire

- * 15. Dr. A.G. Gornall, Committee on Dental Research
Department of Pathological Chemistry,
University of Toronto,
Toronto, Ont. Chairman 31 March, 1962
16. Dr. C.P. Leblond,
Department of Anatomy,
McGill University,
Montreal, P.Q. 31 March, 1963
17. Dr. A.J. Rhodes,
School of Hygiene,
University of Toronto,
Toronto, Ont. 31 March, 1963
18. Dr. R.L. deC.H. Saunders,
Department of Anatomy,
Dalhousie University,
Halifax, N.S. 31 March, 1961
- * 19. Dr. G.D. Scott,
Department of Physics,
University of Toronto,
Toronto, Ont. 31 March, 1963
20. Dr. R.J. Slater,
Research Institute,
Hospital for Sick Children,
Toronto, Ont. 31 March, 1963
21. Miss D.J. Wright, Secretary
National Research Council,
Ottawa 2, Ont. - - -

* Member of the Executive

INITIAL DISTRIBUTION

(i) To members of the Associate Committee on Dental Research

- | | |
|---------------------------------------|---|
| 1. Dr. K. J. Paynter, <u>Chairman</u> | 12. Dr. C. P. Leblond |
| 2. Brig. K. M. Baird | 13. Dr. J. W. Neilson |
| 3. Dr. L. F. Belanger | 14. Dr. G. Nikiforuk |
| 4. Dr. H. K. Brown | 15. Dr. A. J. Rhodes |
| 5. Dr. C. Castaldi | 16. Dr. R. L. deC. H. Saunders |
| 6. Dr. A. D'Iorio | 17. Dr. G. D. Scott |
| 7. Dr. R. F. Farquharson | 18. Dr. R. J. Slater |
| 8. Dr. L. E. Franci | 19. Dr. E. W. R. Steacie |
| 9. Dr. A. G. Gornall | 20. Dr. D. L. Thomson |
| 10. Dr. L. A. Kilburn | 21. Miss D. J. Wright, <u>Secretary</u> |
| 11. Dr. I. Kleinberg | |

(ii) To other persons:

22-26 Deans of Dental Schools (who are not members of the Committee)

- | | | |
|---------------|---|-------------------|
| 22. Alberta | - | Dr. H. R. MacLean |
| 23. Dalhousie | - | Dr. J. D. McLean |
| 24. McGill | - | Dr. J. McCutcheon |
| 25. Montreal | - | Dr. P. Geoffrion |
| 26. Toronto | - | Dr. R. G. Ellis |

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NATIONAL RESEARCH COUNCIL OF CANADA

PROCEEDINGS
OF THE
TWENTY-EIGHTH MEETING
OF THE
ASSOCIATE COMMITTEE ON
DENTAL RESEARCH

OTTAWA

1 - 2 MARCH 1962

ASSOCIATE COMMITTEE ON DENTAL RESEARCH

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ASSOCIATE COMMITTEE ON DENTAL RESEARCH

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I. Attendance:

Dr. R. J. Paynter, Chairman

Dr. L. F. Belanger

Dr. C. C. Caldwell

Dr. R. F. Farquharson

Dr. L. E. Francis

Dr. A. G. Gurnall

Dr. L. A. Killcare

Dr. I. Kleinberg

Dr. G. P. Lohmand

Dr. J. P. Lussier

Dr. J. W. Neilson

Dr. A. J. Rhodes

Dr. E. L. deC. H. Saunders

Dr. G. D. Scott

Col. G. B. Shillington

Mr. A. D. Armstrong, Secretary

Regrets at their inability to attend were received from:

Dr. G. Nikitrus

Dr. D. L. Thomson

The Chairman extended a welcome to Dr. J. P. Lussier who was the only new member of the Committee. He also introduced Col. G. B. Shillington who was representing Dr. R. M. Reid, Director General of Dental Service, Royal Canadian Dental Corps, Department of National Defence.

Summary of the Twenty-Seventh Meeting

The minutes of the Twenty-Seventh Meeting had been distributed to all members and were therefore taken as read.

NATIONAL RESEARCH COUNCIL

Proceedings

of the

Twenty-Eighth Meeting

of the

ASSOCIATE COMMITTEE ON DENTAL RESEARCH

1 - 2 March 1962

The first session convened in the Board Room of the National Research Council, Ottawa, at 9:30 a.m., 1 March 1962.

1. Attendance:

Dr. K. J. Paynter, Chairman
Dr. L. F. Belanger
Dr. C. Castaldi
Dr. R. F. Farquharson
Dr. L. E. Francis
Dr. A. G. Gornall
Dr. L. A. Kilburn
Dr. I. Kleinberg
Dr. C. P. Leblond
Dr. J. P. Lussier
Dr. J. W. Neilson
Dr. A. J. Rhodes
Dr. R. L. deC. H. Saunders
Dr. G. D. Scott
Col. G. B. Shillington
Mr. A. D. Armstrong, Secretary

Regrets at their inability to attend were received from:

Dr. G. Nikiforuk
Dr. D. L. Thomson

The Chairman extended a welcome to Dr. J. P. Lussier who was the only new member of the Committee. He also introduced Col. G. B. Shillington who was representing Brig. K. M. Baird, Director General of Dental Service, Royal Canadian Dental Corps, Department of National Defence.

2. Minutes of the Twenty-Seventh Meeting

The minutes of the Twenty-Seventh Meeting had been distributed to all members and were therefore taken as read.

It was MOVED by Dr. J. W. Neilson and SECONDED by Dr. I. Kleinberg

THAT the Minutes be approved.

CARRIED

3. Business Arising out of the Twenty-Seventh Meeting

(a) Treatment Experimental Animals (Proc. 27th meeting, item 3(d))

The Chairman stated that the preparation of a Canadian code for the treatment of experimental animals was in progress through the Canadian Federation of Biological Sciences, and should be published shortly.

(b) Reports from Grantees (Proc. 27th meeting, item 7)

It was re-confirmed that the practice of having the grantees' reports reproduced as appendices to the Proceedings of the meetings be continued. It was felt, however, that a one-page summary only of the report should be sent to all members of the Committee prior to the meeting. Reviewers would, of course, continue to receive the full report on any project for which a renewal application had been submitted.

(c) The Chairman presented the following report on Summer Undergraduate Assistantships:

Summer Undergraduate Assistantships

In view of the discussion in the Committee at the last meeting dealing with undergraduate Summer Assistantships, the following report has been prepared.

At the 23rd meeting of the Committee in March 1957, the suggestion of providing support for dental students while working in research laboratories during the summer, was first discussed. The following is the minute pertaining to this item: -

"14. Summer Employment for Dental Students

Dr. Lussier asked the Committee to consider the feasibility of offering summer Scholarships to undergraduate dental students, with a view to arousing their interest in research. He felt that although support is being given at the graduate level in the form of grants and Fellowships, this had little effect on the stimulation of interest in research in undergraduates. Dr. Lussier suggested that summer Studentships valued at from

\$600 - \$800 be awarded to one student from each of the five dental schools, to enable them to work in research laboratories between academic sessions. He further suggested that at the end of the summer, the students be asked to submit a brief report of their work and on the department in which they had worked, and a similar report be obtained from the departments concerned on the students. Dr. Lussier felt that in this way undergraduate dental students might be made aware of the opportunities offered in research, opportunities which they can rarely see under the present circumstances of relative inactivity in research evident in most dental schools.

Considerable discussion ensued. Dr. Beveridge felt that the proposed stipends were not realistic, since they would not offer sufficient attraction to offset the advantages of other employment possibilities open to the students. Dr. Copp felt that the idea was very sound and represented one of the best ways of stimulating research in the field of dentistry. He was of the opinion that the best students in the class should be encouraged to participate in such a scheme, and that this would be possible if the remuneration were sufficiently attractive. Dr. Williams suggested that rather than confining the plan to one student from each dental school, a total of five students might be given awards, regardless of their schools.

Dr. Marshall said that such studentships would be outside the scope of the National Research Council at the present time, since the Council makes awards only at the postgraduate level. He suggested, however, that the plan might be operated as an employment opportunity, on a basis similar to that now in operation at the Council in order to recruit people for its own laboratories, and with comparable rates of pay, e.g. \$265 per month initially with nominal increases allowed for experience.

It was the opinion of the Committee that such employment might be offered to dental undergraduates regardless of the amount of academic training they had received, because the earlier one can interest a student in research the greater the likelihood of his continuing interest in it. It was suggested that from the administrative standpoint such students might for the time being, most feasibly be employed in the laboratories of the Committee's Grantees, who could if necessary be given supplementary grants to cover the necessary expenditure involved.

It was moved by Dr. Lussier and seconded by Dr. Beveridge that the Executive be directed to explore the possibilities of employing undergraduate dental students in the laboratories of dental grantees during the summer months and be empowered to act thereon if possible.

Carried."

This minute, I think, very adequately explains the basic purpose for which these awards were instituted,- that is recruitment for dental research. It also explains how the rates of pay were established.

In order to compare these rates with those provided to other groups by the National Research Council, those by the Medical Research Council, and by other organizations, is shown in Table I.

For further comparison, the following list shows the current salary scale for technical personnel at the University of Toronto:-

1. Laboratory Attendant	\$2,300 - \$2,400
2. Laboratory Assistant	2,500 - 2,800
3. Junior Technician	2,950 - 3,300
4. Technician	3,450 - 3,900
5. Senior Technician	3,950 - 4,350
6. Chief Technician	4,600 - 5,100

The National Research Council Dental Summer Assistantships have, in most institutions, become almost a form of Scholarship. Certainly in the University of Toronto, those who are selected to apply for the awards are chosen from a large number of students on the basis of academic performance.

Initially, the salaries paid the dental undergraduate were the same as those paid other undergraduate students for employment in National Research Council laboratories. It should be noted that as of this year the National Research Council rates have gone up, so that students, in their final summer as undergraduates, will be receiving \$315 per month instead of \$305 in National Research Council laboratories.

The dental undergraduate grants are less than those awarded through the Medical Research Council Scholarships, which provide \$1,000 for three months employment to young medical graduates who have achieved scholastic distinction.

Grantees of the Medical Research Council may apply for a sum up to \$800 as a regular part of their grant to employ undergraduate students. In this case, the Council has nothing to do with establishing the monthly rate of pay which are determined by the grantee. These rates usually vary with the institution.

The figures from Halifax were also included for comparison, and they show salaries paid undergraduate dental students at Dalhousie University who are employed in the field of Dental Public Health during the summer months. In addition to this, these students are provided with board and room at no cost to themselves during their period of employ, so that these figures should be modified upward and probably could be

TABLE I
RATES OF PAY FOR STUDENTS

TYPE OF STUDENT														
Year of Employment	N.R.C. U/G Dent.		N.R.C. U/G Reg.		C.D.A. Studentships		M.R.C. Scholarship		M.R.C. Reg. Grant		D.P.H. -H'LFX U/G		GRADUATE N.R.C.	
	RATE		RATE		RATE		RATE		RATE		RATE*		RATE	
	/Mo. /Yr.		/Mo. /Yr.		/Mo. /Yr.		/Mo. /Yr.		/Mo. /Yr.		/Mo. /Yr.		/Mo. /Yr.	
1	265	3180	265	3180	265	3180	333	4000	200	2400	170	2040	145	1740
2	285	3420	285	3420	285	3420	333	4000	200	2400	195	2220	180	2160
3	305	3660	315	3780	305	3660	333	4000	200	2400	200	2400	180	2160

* Plus living costs

somewhat comparable to those apparently paid undergraduate dental students through our grants.

The amounts paid graduate students on National Research Council and Medical Research Council grants are shown in the last column of the table and these are less than those paid undergraduates. It should be appreciated, however, that the graduate student is earning academic credit during his period of employment, which the undergraduate is not.

Canadian Dental Association Studentships, which are granted for the same purpose as National Research Council undergraduate Assistantships have been established at the same rate as those of this Committee.

I think there is general agreement in the schools that these grants are very popular, and form an additional academic prize for undergraduate dental students. Data is gradually accumulating from which a measure of the success of the plan can be estimated. Of the total number of students who have received these awards since they were first initiated, and who have now graduated and thus had an opportunity to have become dental teachers, from one-quarter to one-third appear to have done so.

At the meeting of the Interdepartmental Medical Research Co-ordinating Group held in October 1961, considerable discussion was given over to undergraduate student support during the summer. It was generally agreed by the group that students should receive reward for each successive year of employment as is done through our own grants, and the opinion was expressed by a number of those in attendance at the meeting that the importance of these awards in terms of recruitment could not be over-estimated. It was suggested that students might even obtain credit towards a B.Sc. degree during their period of employment and in Manitoba, medical students are permitted to do this. A number of national granting bodies, such as the Muscular Dystrophy Association, the National Cancer Institution, etc., do also provide funds for undergraduate support.

I think it is reasonable to conclude that our summer undergraduate Assistantships are serving a very valuable purpose in providing experience for academically well qualified undergraduate dental students to obtain an experience that is otherwise unavailable to them in research, with a view to recruiting them into research and teaching on graduation. Figures available to date, indicate that the plan is being met with considerable success.

The fact that there is competition for these awards in terms of academic record would put them on a basis comparable to Medical Research Council Scholarships rather than to those grants made routinely through Medical Research Council Grants-in-Aid.

After an extensive discussion it was MOVED by Dr. J. P. Lussier and SECONDED by Dr. R. L. deC.H. Saunders

THAT because of the serious administrative problems that have developed as the undergraduate Summer Assistantship programme has grown, the present programme be discontinued, and THAT a new category of award - the Summer Undergraduate Dental Research Scholarship be established. These Scholarships will be valued at \$1,000 each, and will be awarded two to each Canadian dental school for employment of undergraduate dental students for a three-month period in a research laboratory during the summer months. Winners of the awards will be chosen by the Dean of the Faculty in consultation with members of his staff, on the basis of academic ability and interest in research.

CARRIED

It was MOVED by Dr. A. G. Gornall and SECONDED by Dr. L. E. Francis,

THAT undergraduate dental students other than the winners of Scholarships, could be employed by grantees of this Committee at rates not exceeding \$900 for a three-month period. Application for this support should be included as part of the regular grant application.

CARRIED

(d) General Research Grants to Deans (Proc. 27th meeting, item 16, p. 23)

The Chairman stated that since there was no encouragement from the National Research Council he felt that it was inadvisable at this time to attempt to establish a General Research Grant to the Deans of dental schools.

(e) Chairman, Council on Dental Education (Proc. 27th meeting, item 17, p. 23 - Council action)

The recommendation that the Chairman of the Canadian Dental Association's Council on Dental Education be appointed an ex-officio of the Associate Committee on Dental Research was not approved

by the Council on the grounds that it might prove to be a poor policy for the future. The Council felt that it would be more appropriate to issue invitations on an annual basis in order to provide flexibility for the Committee.

4. Chairman's Remarks

- (a) Introduction
- (b) Personnel
- (c) Canadian Conference on Dental Research
- (d) Interdepartmental Medical Research Co-Ordinating Group
- (e) Canadian Dental Research Foundation
- (f) Summary

INTRODUCTION

Two years ago you will recall, at the 1960 meeting, this Committee found itself in the unenviable position of having applications for financial aid far in excess of available funds. Last year at our request the National Research Council doubled our allocation. However, insufficient applications were received to spend this extra money and about \$60,000 was returned to the general funds of the Council. One of the main reasons given for the lack of requests last year was disappointment the previous year, and fears for similar disappointment again. In the meantime the schools have been informed of the availability of money, but again this year the great increase in applications that we expected has not come. I would like to make a few comments on dental research spending in Canada with a view to considering how this Committee can do more to encourage growth.

It might be of interest to compare Canadian figures with those of the United States, where a very active dental research programme is going on.

In the United States this year through the National Institutes of Health, approximately \$12,000,000 will be spent in dental research. I estimate that the total amount spent in Canada for the same purpose will be approximately \$260,000, of which about \$140-\$180,000 will be provided by the National Research Council. This latter figure is the one that should be compared with the United States' \$12,000,000. It might also be of interest to note that \$12,000,000 is almost the same as that being spent to support all medical and dental research in this country this year. There are approximately 100,000 dentists in the United States, and about 6,000 in Canada. On a per capita dentist ratio, we would be spending about \$720,000 (compared with \$180,000).

But in addition to the differences in the gross amount spent for dental research in the U.S.A. and Canada, in each division under which grants are made we also suffer by comparison. For example:

Fellowships

<u>N.I.H.</u>	<u>N.R.C.</u>
1st - 3rd years - \$5,000 - \$6,000	Up to \$5,000
plus:	
\$500 per dependent	Nil
Tuition	Nil
Travel at 8¢ per mile	Nil
\$500 supply grant to institute	Nil

Of course, we are not necessarily wrong in offering less money for Fellowships grants than the United States is doing. However, since most of our Fellows have taken their studies in American institutions they work shoulder to shoulder with N.I.H. Fellows, and they are financially embarrassed by comparison. Recently I have been provided with estimates of the basic cost of living in Boston (not including books, clothing, entertainment, cleaning, laundry, etc.). For a single man the estimate was \$2,515.00 per year, and for married man with a family \$3,885.00. In all instances, costs for medical insurance, food, apartment rent, home insurance, automobile insurance, etc., are considerably higher than in the Toronto area. If the cost for books and tuition is added to the estimated basic cost, it can be readily seen that a grant of \$4,000 - \$5,000 is not really adequate.

Grants-in-Aid

In 1960, N.I.H. grants-in-aid of dental research averaged \$13,500 per worker. In 1961 the average grant made by this Committee was \$4,000 - less than one-third the U.S. average for the previous year. It surely cannot be that much less expensive to do research in this country.

General Grants to Deans

This year the National Institutes of Health will make a general research grant of at least \$25,000 to the Dean of each American dental school to use as he sees fit to support and encourage research in his school. In Canada the Deans now receive no direct support from the National Research Council, although in some instances they have received some help through the National Research Council general research grants to University Presidents. One might question whether sums relatively as large as those available in the United States could be wisely spent here, nevertheless when the itemized differences as great

as these exist perhaps we should reconsider what we are doing here. We do have to face the fact that, whether we want to be or not, we are in competition with the United States in respect to doing research and to obtaining research workers and teachers for our schools.

PERSONNEL

Our biggest single obstacle to the development of dental research in Canada is still the need for more research personnel. How can we recruit more?

In 1960-61 there were 179 students enrolled in the 4th year of the dental courses in all of the schools in Canada. If we assume that researchers and teachers should come from the top 10 per cent of these, our pool of potential candidates totals 17 or 18 candidates. If the schools were filled to capacity there would still only be 327 graduates each year, providing a potential research pool of about 30-35 candidates. This is the small group on which we should be concentrating.

The summer Assistantships available both through the National Research Council and the Canadian Dental Association are beginning to pay dividends in this respect, and they are probably our best single means of attracting bright students into research. It has been interesting to learn through meetings of the Interdepartmental Research Co-ordinating Group that a number of other national granting agencies who support medical research in the country now recognize undergraduate summer student support as one of their responsibilities. At a meeting of the group last October, considerable time was spent discussing the need and value of these awards.

I have already pointed out that our Fellowships, while among the highest in Canada, still fall far short of similar funds available through the United States. Perhaps we are losing potential candidates because of insufficient support while in training. This Committee should consider recommending that Fellowships be increased in amount.

It has been interesting to read and hear that other granting organizations are now providing or planning to provide the type of personnel support we arranged for last year when we revised our Associateship regulations. The National Institutes of Health have recently established Research Career Development Awards for this purpose, and medical research sponsoring groups in this country are now considering such grants.

Continuing with and, where necessary increasing, any or all of these awards to recruit, train and establish dental research workers will surely help add to the group. However, the provision of funds will not do it all. Research workers themselves can still do a great deal by personally encouraging and guiding prospective candidates.

CANADIAN CONFERENCE ON DENTAL RESEARCH

In October 1961, under the sponsorship of the Canadian Dental Research Foundation and the Council on Research of the Canadian Dental Association, the first Canadian Conference on Dental Research was held in Toronto. The Conference was made possible financially through a gift from the Procter & Gamble Company. At this meeting, which lasted about a day and a half, two or three research workers from each of the Canadian dental schools, and a representative from the Royal Canadian Dental Corps, and others, met to present and to listen to a series of research papers reporting work being conducted in the various institutions across the country. The meeting was a great success, and plans were proposed to make a second Canadian Conference on Dental Research, to be held later this year, probably in Western Canada. The Committee will be asked to give consideration to sponsoring this Conference later in the meeting.

INTERDEPARTMENTAL MEDICAL RESEARCH CO-ORDINATING GROUP

As I have already indicated, a number of matters pertaining to medical and dental research in Canada were considered at the last meeting of the Interdepartmental Medical Research Co-ordinating Group in January of this year. One of these was terminology pertaining to awards. It was suggested that the individual granting organizations should consider reviewing their award terminology, e.g. Fellowships, Associateships, etc. to conform to that used by the Medical Research Council. This could avoid confusion when discussing various grant categories with one another. Those attending the meeting were provided with a booklet produced by the Medical Research Council, which outlines the types of awards offered through the Council, and which is presumably used to publicize Medical Research Council activities. The thought occurred to me that perhaps such a booklet should be prepared for this Committee with a view to drawing more attention to the kinds of research support offered dentistry through the National Research Council.

CANADIAN DENTAL RESEARCH FOUNDATION

The Canadian Dental Research Foundation was established more than forty years ago to sponsor and encourage dental research in Canada, and the funds for its establishment were provided by an appeal made to dentists and dental organizations across Canada, as a memorial to those dentists who had died during the 1914-18 war. The income of the Foundation is distributed by the Council on Research of the Canadian Dental Association, which is also the Board of Directors of the Foundation.

Last year the Board of Governors of the Canadian Dental Association agreed that the Foundation could serve a useful and necessary purpose by acting as a central agency to provide a liaison between the

various organizations supporting dental research in Canada. For this purpose the Foundation will establish a central file at C.D.A. Headquarters where current data pertaining to dental research growth, personnel, etc. will be maintained and available.

In order to expedite this activity, it has been suggested that the President of the Foundation should attend meetings of this Committee. I would like to ask the Committee to consider routinely extending an invitation to the President of the Canadian Dental Research Foundation to do so.

SUMMARY

1. In view of the serious differences that are developing between the amounts available through N. R. C. Dental Fellowships and the costs involved in acquiring a graduate education, I would like the Committee to consider extending our Fellowships to include items such as tuition, travel costs, dependents, allowance, etc.

2. To comply with the suggestion of the Interdepartmental Medical Research Co-ordinating Group, I suggest that the Secretary be requested to review our grant terminology, and to make suggestions for change where necessary so they will conform to the terminology of the Medical Research Council of Canada.

3. I would also like the Committee to consider the possible value of providing a booklet publicizing the research support available through the Committee.

4. Further, I would like to recommend that the President of the Canadian Dental Research Foundation be regularly invited to attend meetings of the Committee. This will not only provide the Foundation with first-hand information about the Committee's activities, but will also ensure that the Committee is kept up-to-date as to dental research activities going on elsewhere in Canada.

Following the Chairman's remarks Dr. R. F. Farquharson discussed various aspects of the role played by the Medical Research Council in assisting and promoting medical research. The Committee then examined the Medical Research Council extramural programme booklet which was put out in 1961 explaining the methods used by the Medical Research Council in its operations.

After a discussion of the ways in which the Committee could improve its liaison with the Dental Schools and dental students, it was MOVED by Dr. R. L. deC.H. Saunders and SECONDED by Dr. J. P. Lussier

THAT a brochure explaining the National Research Council's dental awards be prepared for circulation.

CARRIED

Further to this it was MOVED by Dr. C. Castaldi and SECONDED by Dr. A. G. Gornall

THAT the terminology of grants, Fellowships, etc., by the Committee be altered where necessary to conform to that employed by the Medical Research Council.

CARRIED

In discussing assistance to students in general, it was decided that at present the Council would not provide for the payment of tuition if special cases of this type arose.

The Committee then recommended that the following schedule be used for the payment of Fellowships to dental students:

<u>YEARS OF GRADUATE STUDY</u>	<u>RATE</u>
First year	\$3,500 - \$4,000
Second year	4,000 - 4,500
Third year	4,500 - *5,000
+ \$500 per year for marriage allowance	
* Ceiling of \$5,000	

5. Executive Action 1961-62

(a) Name of Applicant: POYTON, H. G.

Request: That since money awarded under DA-96 in the amount of \$475.00 for purchase of a Photovolt electronic densitometer was no longer needed for this equipment due to funds from another source, this sum, \$475.00, should be used to purchase other items of equipment such as varying range dosimeters, a charging unit, etc.

Decision: Request granted

(b) Name of Applicant: SOLOMONS, C. C.

Short Title of Proposed Research: "Studies on the Chemistry of Collagen and Calcification"

Salaries of Graduate Student Assistants	\$2,400.00	No. 1
Equipment, Materials and Supplies	2,500.00	
TOTAL	4,900.00	

Decision: Request granted in the amount of \$3,500.00 Annual Grant DA-97

(c) Name of Applicant: FINDLAY, J.

Short Title of Proposed Research: "An Investigation into the Effect of Hypophysectomy and Hormone Replacement Therapy on the Oral Tissues of Female Rats"

Request: (1) That supplement of \$404.00 for Equipment, Supplies, etc., be added to Grant DA-87.
(2) Major Equipment Grant of \$702.50 for a special table and an electric motor to provide power for a Jung Microtome.

Decision: Request granted in the amount of \$1106.50 as a supplement under annual grant DA-87.

(d) Name of Applicant: HUNTER, H. A.

Request: That \$1030.00 be provided under a Major Equipment Grant to purchase a Norelco Contact Microradiographic Unit.

Decision: Request granted in the amount of \$1030.00 under annual grant DA-98.

(e) Name of Applicant: LISTGARTEN, M. A.

Request: That a supplement of \$140.00 be granted to his Graduate Dental Research Fellowship of \$3750.00.

Decision: Request not granted.

(f) SUMMER ASSISTANTSHIP

Name of Applicant: PUCHINGER, L. McGill University. First in class. Recommended by Dr. L. E. Francis and Dr. McCutcheon.

Due to the inability of R. Gibbons to take up his Summer Assistantship, it was proposed by Dr. L. E. Francis that his position be filled by L. Puchinger.

Decision: Request granted.

(g) ASSOCIATESHIP

Name of Applicant: PRITCHARD, E. T.

Decision: Associateship granted in the amount of \$8300.00. Due to Dr. E. T. Pritchard being a Fellow of the National Multiple Sclerosis Society at Harvard University during 1961-62, he requested that his Associateship start 1 July 1962. Request to be reviewed by Committee.

It was MOVED by Dr. C. P. Leblond and SECONDED by Dr. L. E. Francis

THAT Executive Actions taken during 1961-62 be approved.
CARRIED

6. Reports from Grantees

Seventeen grantees submitted progress reports on the work on which they had been engaged during 1961-62.

Four grantees presented reports orally at the annual meeting.

<u>Name</u>	<u>No.</u>	<u>Title of Project Report</u>
Leblond, C. P. Anatomy McGill	DA-23	"Entry of Carbohydrates and Proteins into Teeth as shown with the Help of Radioactive Elements"
Lussier, J. P. Faculty of Dentistry Montreal	DA-70	"The Biosynthesis of Citrate in Bone Tissue under the influence of Vitamin D and related Conditions"
Paynter, K. J. Faculty of Dentistry Toronto	DA-72	"Factors Influencing Tooth Morphology and Caries Susceptibility"
Francis, L. E. Oral Pathology McGill	DA-88	"Further Studies in Tooth Transplantation" (The development of an "in-vivo" extra-oral site for growth of the developing third molar to be used in conjunction with tooth bank studies)

7. Meeting of the Executive

The Chairman informed the Committee that the members of the Executive (Dr. A. G. Gornall, Dr. I. Kleinberg, Dr. J. P. Lussier, in addition to the Chairman) had met on the afternoon of 26 February 1962, at 2:30 p.m. Preliminary consideration had been given to the applications for Associateships, for Fellowships and for grants in aid; the recommendations of the Executive were submitted to the Committee as each application was reviewed.

8. Financial Status of the Committee

The Secretary informed the Committee that up to \$182,000 could be made available to the Committee for 1962-63; of this total,

\$30,000 was encumbered for term grants previously approved, and \$2,000 was encumbered for administration purposes.

9. Applications for Associateships

One application for a Dental Research Associateship tenable during 1962 to 1964 was considered. Action was taken as follows:

Pritchard, E. T.	To work in the Dept. of Oral Biology, Faculty of Dentistry, University of Manitoba	APPROVED \$8,700 plus \$3,000 Operating Grant, plus \$700.00 payment of applicant's share of cost of pension and other benefits in which university staff members normally participate.
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10. Applications for Fellowships

Eight applications for Dental Research Fellowships tenable during 1962-63 were considered: three for renewal of current Fellowships and five for a first award. Action was taken as follows:

Heppler, L. J.	To work in the Dept. of Oral Biology, Faculty of Dentistry, University of Manitoba, under Dr. J. Kleinberg	APPROVED \$4,500
Johnson, R. H.	To work in the Dept. of Oral Diagnosis and Oral Medicine, University of Indiana, under Dr. D.F. Mitchell	APPROVED \$4,000
Johnston, M. C.	To work in the Dept. of Dentistry, Dental Research, and Dept. of Anatomy at the University of Rochester, under Dr. E. Johansen	APPROVED \$5,000
Listgarten, M. A.	To work in the Dept. of Oral Pathology, at the Harvard School of Dental Medicine, under Dr. P. Goldhaber	APPROVED \$4,500

Sandham, H. J.	To work in the Dept. of Oral Biology, Faculty of Dentistry, University of Manitoba, under Dr. I. Kleinberg	NOT APPROVED
Taichman, N. S.	To work at the Harvard School of Dental Medicine, under Dr. P. Goldhaber	APPROVED \$5,000
Teteruck, W. R.	To work in the Dept. of Dental Materials at the Indiana University, under Prof. R. W. Phillips	NOT APPROVED
Weinstock, A.	To work at the Harvard School of Dental Medicine	NOT APPROVED

It was MOVED by Dr. J. P. Lussier and SECONDED by Dr. C. Castaldi

THAT payment of these grants be made as outlined in the above schedule.

CARRIED

11. Applications for Grants, 1962-63

The Chairman noted that funds were already committed for the coming year for Term Grants as follows:

Copp, D. H.	Phosphorus deficiency:	\$6,400
Physiology,	Effects on bones and teeth	The second instal-
Br. Columbia	DT-59	ment of a three-year Term Grant
Francis, L. E.	Studies on gingival changes	\$5,000
Oral Pathology,	due to drug administration	The third instalment
McGill	DT-76	of a three-year Term Grant (see p. 19)
Grainger, R. M.	Statistical consultation and	\$7,000
Dental Research,	analysis unit	The second instal-
Toronto	DT-89	ment of a three-year Term Grant
Kleinberg, I.	1. Further studies on the	\$6,600
Biochemistry,	role of the dental plaque in	The second instal-
Manitoba	the caries process.	ment of a three-year
	2. Further studies on the	Term Grant

	effect of substances removed during the refining of carbohydrates on calcium phosphate solubility DT-78	
Nikiforuk, G. Dental Research Toronto	Organic compounds of dental tissue DT-84	\$5,000 The second instalment of a three-year Term Grant (see p. 21)

Twenty-six grant applications were then considered. Many of them had been reviewed before the meeting by scientists qualified in the particular field involved, and copies of the reviewers' comments were made available to all members of the Committee.

<u>Applicant</u>	<u>Title and Amount Requested</u>	<u>Recommendation</u>
Anderson, D.L. Dental Research, Toronto	Histochemical investigation of cell cytoplasm and inter-cellular interfibrillar material DA-86 \$3,000	Approved \$3,000
Baril, C. Orthodontics, Montreal	Electromyography of muscles innervated by the Vth and VIIth cranial nerves DA-107 \$5,500	Approved \$5,500
Dale, J.G. Dental Research Toronto	The study of bone resorption by implantation methods DA-99 \$3,800	Approved \$3,800 (Student assistant requested for Summer Assistantship)
Donohue, W.B. Oral Pathology, Montreal	Glycogen content and distribution in young and aged buccal mucosa DA-100 \$3,980	Approved \$3,980 (Student assistant requested for Summer Assistantship)
Dowse, C.M. Oral Biology, Manitoba	Metabolism of periosteum DA-81 \$8,400	Approved \$8,400 (Student assistant requested for Summer Assistantship)
Findlay, J. Periodontics, Dalhousie	An investigation into the effects of an artificially induced oestrus cycle on the periodontal tissues of hypophysectomised rats DA-87 \$3,616	Approved \$900.00 (2 Student assistants requested for Summer Assistantships)

<u>Applicant</u>	<u>Title and Amount Requested</u>	<u>Recommendation</u>
Francis, L.E. Oral Pathology, McGill	Further studies in tooth transplantation (the development of an "in-vivo" extra-oral site for growth of the developing third molar to be used in conjunction with tooth bank studies) DA-88 \$2,000	Approved \$2,000 (Student assistant requested for Summer Assistantship. Cost to be added to DT-76, see p. 17)
Hunt, A.M. Dental Research, Toronto	Measurement of strontium ⁹⁰ and carbon ¹⁴ in primary teeth of Canadian children DA-82 \$6,700	Approved \$6,500
Kasloff, Z. Restorative Dentistry, Manitoba	The relationship of defects in teeth associated with induced stresses to the histological pattern of tooth structure DA-90 \$5,450	Approved \$5,000
Khairat, O. Bacteriology and Immunology, Manitoba	Post-extraction bacteremia with special reference to anaerobes and antibiotic protection against subsequent development of subacute bacterial endocarditis DA-101 \$3,754.65	Approved \$3,750
Kleinberg, I. Oral Biology, Manitoba	1. Beckman DU spectrophotometer, power supply, flame attachment, recorder and other accessories and labour necessary to build an Ultra-micro Flame Spectrophotometer covered by U.K. Pat. Appln. No. 1240/58 2. Spinco electrophoresis cell (Durrum type), Duostat power supply and equipment kit DE-102 \$5,747.85	Approved \$5,750

<u>Applicant</u>	<u>Title and Amount Requested</u>	<u>Recommendation</u>
Kleinberg, I. Oral Biology, Manitoba	1. Further studies on the role of the dental plaque in the caries process. 2. Further studies on the effect of substances removed during the refining of carbohydrates on calcium phosphate solubility DT-78 \$2,240	Approved \$2,000 (2 Student assistants requested for Summer Assistantships) (Supplement for DT-78)
Kropp, B.N. Histology and Embryology, Queen's	An investigation of the effect of salivary gland extract, saliva and sialagogues on growth of teeth DA-103 \$4,750	Approved \$1,750
Kropp, B.N. Histology and Embryology, Queen's	The influence of fluoride on differentiation of calcified structures and on teratogenesis DA-91 \$3,475	Approved \$3,400
Leblond, C.P. Anatomy, McGill	Entry of carbohydrates and proteins into teeth as shown with the help of radioactive elements DT-23 \$5,000	Approved \$5,000 as three-year Term Grant (3 Student assistants requested for Summer Assistantships)
Lussier, J.P. Physiology, Montreal	The biosynthesis of citrate in bone tissue under the influence of vitamin D and related conditions DA-70 \$4,800	Approved \$4,800 (Student assistant requested for Summer Assistantship)
Marshall, F.J. Endodontology and Oral Histology, Manitoba	Autoradiographic studies of dentine permeability using radioactive nickel ⁶³ tracers DA-83 \$4,745	Approved \$2,000

<u>Applicant</u>	<u>Title and Amount Requested</u>	<u>Recommendation</u>
Nikiforuk, G. Dental Research, Toronto	Automatic camera attachment "Orthomat" including one cassette Binocular photo tube etc. Two eyepieces PO 10x etc. Ground glass plate in mount Focusing magnifier 5 spare film cassettes DE-104 \$2,550	Approved \$2,550 (Student assistant re- quested for Summer Assistantship. Cost to be added to DT-84, see p.18)
Paynter, K.J. Dental Research, Toronto	Factors influencing tooth morphology and caries susceptibility DT-72 \$9,000	Approved \$8,000 as three-year Term Grant (Student assistant re- quested for Summer Assistantship)
Saunders, R.L.deC.H. and Rockert, H. Anatomy and Histology, Dalhousie	An X-ray microscopy study of the vascularization of the human dental pulp at various ages DA-105 \$1,050	Approved \$1,050
Sinclair-Hall, A.H. Anatomy, Manitoba	Polyvinyl-alcohol sponge and the healing of bony defects DA-92 \$2,000	Approved \$1,200
Spouge, J.D. Oral Biology, Manitoba	An investigation into the rela- tive roles of intermittent lateral stresses applied to the teeth, and administration of a soft but nutritionally adequate diet, in the production of perio- dental disease in the jaws \$1,372.50	Not approved
Trott, J.R. Periodontology, Manitoba	An histological investigation into the development of the periodontal membrane in rats and mice DA-85 \$3,100	Approved \$1,000 (Student assistant re- quested for Summer Assistantship)
Tuba, J. Biochemistry, Alberta	Role of dietary phosphates in dental caries DA-94 \$10,000	Approved \$7,500 (Student assistant re- quested for Summer Assistantship)

<u>Applicant</u>	<u>Title and Amount Requested</u>	<u>Recommendation</u>
Tuba, J. Biochemistry, Alberta	One - Cary Model 15 Spectrophotometer One - Thermostatable Absorption Cell Compartment DE-106 \$11,700	Approved \$4,000
Youdelis, W. V. Mining and Metallurgy Alberta	Fundamentals of amalgam metallurgy and development of dental amalgams DA-95 \$5,395	Approved \$3,840 (Student assistant requested for Summer Assistantship)

In summary, a total of twenty-five applications amounting to \$123,126 were considered. Twenty-six grants amounting to \$96,670 were recommended.

It was MOVED by Dr. Kleinberg and SECONDED by Dr. Lussier

THAT grants be made in the amounts specified.

CARRIED

During the discussion of the applications for those grants which were requested by attending members of the Committee, each committee member left the room until his case had been discussed and the recommendation had been made.

The Committee decided that under normal conditions an applicant should have been the recipient of three single grants before being recommended for a term grant.

12. Application for Support for Canadian Conference on Dental Research

The Organizing Committee of the 1962 Canadian Conference on Dental Research (a subcommittee of the Council on Research of the Canadian Dental Association) submitted to the Associate Committee on Dental Research a request for \$4,500.00 for the support of a meeting to be held at Banff, Alberta. One of the purposes for the meeting is to explore the possibility of forming a Canadian Dental Research Association.

The formal request explained that a precedent had been set by a National Research Council body in that the Medical Research Council provides an annual grant in support of the meeting of the Western Regional Group.

The meeting would be for a period of about two days, and would be attended by five or six representatives from each of the Canadian Dental Schools and representation from other Canadian organizations doing dental research. The meeting will be held probably in the fall of 1962.

An amended request was submitted during the meeting of the Associate Committee on Dental Research for \$1,500.00. It was explained that the Procter and Gamble Company, which had sponsored the first meeting held in 1961, would provide \$3,000.00 towards support of the 1962 meeting.

During the discussion on the request and its amendment, the Associate Committee on Dental Research stressed its strong support for a meeting of this kind. The Committee felt that the number of dental research people who were to be invited should be increased in number, and voted financial support with that desire in mind.

It was MOVED by Dr. C.P. Leblond and SECONDED by Dr. A.G. Gornall

THAT a grant of \$3,000 be made for the support of the 1962 Canadian Conference on Dental Research.

CARRIED

13. Summer Dental Research Assistants

Applications this year had been submitted directly to a Committee representative in each of the various dental schools: after preliminary screening in the schools, the names of those who were judged most suitable, and could be accommodated by a grantee, were submitted to the Committee for consideration.

It was decided by the Committee that in future the applicants for grants should be free to hire such students as they wished subject to the requirements of the dental schools in which the students are studying. The salary for these summer assistants should be in line with salaries paid by the University for similar types of work with a ceiling which is to be decided by the Associate Committee on Dental Research at its annual meeting. The applicant should include cost of employing such summer assistants in his application for a grant.

It was decided by the Committee that the secretary should inform the dental schools of this change in the regulations.

After review of the individual cases, it was AGREED that the following Assistants be appointed for the coming summer, and the amounts

of their salaries be added to the 1962-63 grants of the designated supervisors:

Alberta

Blackett, J.A.	To work with Dr. W.V. Youdelis	3 mos. at \$285 \$855
Henderson, P.D.	To work with Dr. J. Tuba	3 mos. at \$285 \$855

Dalhousie

MacIntosh, D.	To work with Dr. J. Findlay	3 mos. at \$265 \$795
Daly, C.P. or Taylor, T.	To work with Dr. J. Findlay	3 mos. at \$305 \$915

Manitoba

Chesko, E.	To work with Dr. C.W. Dowse	3 mos. at \$285 \$855
Kaufman, H.W.	To work with Dr. I. Kleinberg	3 mos. at \$305 \$915
Mollot, M.	To work with Dr. I. Kleinberg	3 mos. at \$305 \$915
Peikoff, M.D.	To work with Dr. J.R. Trott	3 mos. at \$305 \$915

Montreal

Albert, G.	To work with Dr. W.B. Donohue	3 mos. at \$305 \$915
Ambrozaitis, J.	To work with Dr. J.P. Lussier	3 mos. at \$285 \$855

McGill

Giacomelli, J.F.	To work with Dr. L.E. Francis	3 mos. at \$265 \$795
Gibbons, R.	To work with Dr. C. Leblond	3 mos. at \$285 \$855
Lander, P.	To work with Dr. C. Leblond	3 mos. at \$285 \$855
Puchinger, L.	To work with Dr. C. Leblond	3 mos. at \$285 \$855

Toronto

Bing, H.R.E.	To work with Dr. G. Nikiforuk	2 mos. at \$305 \$610
Clarke, A.J.	To work with Dr. R.M. Grainger	3 mos. at \$285 \$855
Direnfeld, V.N.	To work with Dr. J.G. Dale	2 mos. at \$305 \$610
Esilman, G.	To work with Dr. R.M. Grainger	3 mos. at \$285 \$855
Mills, W.H.	To work with Dr. K.J. Paynter	3 mos. at \$285 \$855
Wong, W.	To work with Dr. A.M. Hunt	2 mos. at \$305 \$610

14. Allotment for 1962-63

The Committee allotment for 1962-63 was allocated as follows:

1 Associateship	\$ 12,400
5 Fellowships	23,000
7 Term Grants	43,000
24 Annual Grants	83,670
Canadian Conference on Dental Research	3,000
Administration	2,000
20 Summer Research Dental Assistants	16,545
Total	183,615

15. Visiting Scientists

The Associate Committee on Dental Research of the National Research Council of Canada believes that dental research activities in this country have reached the point that the Council should support visits by distinguished scientists to Canadian dental schools.

Providing of course that they are well organized and thoughtfully planned, the benefits of such visits are obvious, diverse, and multiple. They should be particularly beneficial in a country such as Canada where distances are great, and where consequently, infrequent opportunities exist for anything but the most brief exchange of scientific information on a personal basis.

The Associate Committee notes also that the Medical Research Council of Canada has already recognized this need by establishing awards for visiting scientists to Canadian medical schools and an outline of the medical award is herewith detailed.

VISITING SCIENTISTS

(Apply in the form of a letter before 1 January)

Up to three awards will be made available each year to enable experienced investigators to work in Canadian laboratories. Application may be made on behalf of investigators in the medical sciences from abroad or on behalf of such investigators normally resident in Canada. The awards shall be tenable for periods of not less than three, and not more than twelve months, and will be held from time to time in the various medical schools or their affiliated institutions.

The stipend provided shall be not more than \$600 per month, and will depend on the qualifications and experience of the successful candidates. Travel allowance, and a research allowance pro-rated at \$3,000 per annum, shall be considered in each case.

Applications for these awards shall be made by the host university in the form of a letter giving full details, before 1 January, for consideration at the March meeting of the Council.

.....

In the light of the foregoing circumstances, the Associate Committee on Dental Research recommends that:

a new type of award be established which would support visiting scientists working in Canadian dental schools, and that the terms of this new award conform generally to those set forth by the Medical Research Council in its "Visiting Scientist Award".

.....

A tentative set of terms for the proposed dental award is herewith detailed:

VISITING SCIENTISTS

Up to three awards will be made available each year to enable experienced investigators to work in Canadian laboratories. Application may be made on behalf of investigators in the dental sciences from abroad or on behalf of such investigators normally resident in Canada. The awards shall be tenable for periods of not less than three, and not more than twelve months, and will be held from time to time in the various dental schools.

The stipend provided shall be not more than \$600 per month, and will depend on the qualifications and experience of the successful candidates. Travel allowance, and a research allowance pro-rated at \$3,000 per annum, shall be considered in each case.

Applications for these awards should be made by the host university, in the form of a letter giving full details, before 1 January, for consideration at the March meeting of the Council.

16. Membership of the Committee

The Chairman informed the Committee that two new members should be elected to replace Dr. R. J. Slater and Dr. J. W. Neilson who had retired. He also stated that Dr. L. E. Francis, Dr. I. Kleinberg, Dr. L. F. Belanger and Dr. A. G. Gornall had each completed one three-year term on the Committee and that he himself had completed two three-year terms on the Committee as of 1 April 1962.

It was MOVED by Dr. A. G. Gornall and SECONDED by Dr. A. Rhodes

THAT Dr. W. C. Wilt of the University of Manitoba be recommended to Council for appointment to the Committee for a three-year term terminating 31 March 1965.

CARRIED

It was MOVED by Dr. C. Castaldi and SECONDED by Dr. A. G. Gornall

THAT Dr. R. M. Grainger of the University of Toronto be recommended to Council for appointment to the Committee for a three-year term terminating 31 March 1965.

CARRIED

It was MOVED by Dr. C. P. Leblond and SECONDED by Dr. A. Rhodes

THAT Dr. L. E. Francis, Dr. I. Kleinberg, Dr. L. F. Belanger and Dr. A. G. Gornall be recommended for reappointment to the Committee for further three-year terms, terminating 31 March 1965.

CARRIED

It was MOVED by Dr. C. P. Leblond and SECONDED by Dr. I. Kleinberg

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Granger
Anderson, L. J. DA-86

THAT Dr. K. J. Paynter be recommended for reappointment to the Committee and as Chairman for a further one-year term, terminating 31 March 1963.

CARRIED

Dewar, C. M. DA-87
Francis, J. C.
Hart, A. M.

It was MOVED by Dr. A. G. Gornall and SECONDED by Dr. J. P. Lussier

THAT the Executive should give consideration to appointing a new Chairman or reappointing the present Chairman one year before the present Chairman would retire.

CARRIED

Kasloff, Z. DA-90
Kropp, D. W. DA-91

17. Estimates for 1963-64

It was decided that since there was a steady increase in the number of applications for grants and in the amount of money needed to support dental research in Canada, and since it was anticipated that there would be an increasingly larger number of applicants for Fellowships and probably Associateships, it was suggested that the Committee should submit a request for \$250,000 to carry out its obligations in 1963-64.

Mead, Z. DA-79
Paynter, K. J. DT-72

18. Date of the Next Meeting

Unless the Chairman felt it necessary to call a meeting to consider further applications, the next meeting of the Committee would be held at approximately the same time next year.

Sprague, J. D. DA-93
Trott, J. R. DA-94
Youdella, W. Y. DA-95
Tuba, J. DA-96
Dale, J. G. Special

The meeting adjourned at 2:45 p.m. on 2 March 1962.

Members of the Associate Committee T

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REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

NATIONAL RESEARCH COUNCIL

DECEMBER - 1961

NAME: DR. D.L. ANDERSONINSTITUTION: FACULTY OF DENTISTRY, UNIVERSITY OF TORONTOPROJECT TITLE: DA-86, "HISTOCHEMICAL INVESTIGATION OF CELL
CYTOPLASM AND INTERCELLULAR INTER-
FIBRILLAR MATERIAL"

1. SULPHATED POLYSACCHARIDES AND FIBER FORMATION

INTRODUCTION

SULPHATED MUCOPOLYSACCHARIDES APPEAR TO BE INTIMATELY RELATED TO FIBROPLASIA. THESE COMPOUNDS ARE PRESENT AT EARLY STAGES OF FIBER FORMATION, BUT ARE NOT INCORPORATED WITHIN THE FIBERS. AS JUDGED BY METACHROMATIC STAINING INTENSITY, SULPHATED POLYSACCHARIDE IN WOUND HEALING IS FORMED IN THE FIRST 1 TO 5 DAYS AND RAPIDLY FALLS IN CONCENTRATION FROM THE 6TH TO 9TH DAY (DUNPHY AND UDUPA, 1955). S^{35} INCORPORATION AT A CARRAGEENIN GRANULOMA SITE SUGGESTS EARLY RAPID SYNTHESIS OF SULPHATED POLYSACCHARIDE, HIGH BY THE THIRD DAY, MAXIMAL AT 5 TO 6 DAYS, AND A RAPID DECLINE TO NORMAL LEVELS BY THE 14TH DAY (SLACK, 1957). INCORPORATION OF S^{35} INTO COTTON PELLET GRANULOMATA WAS MAXIMAL FROM 2 TO 6 DAYS AND WAS ASSUMED TO BE PRIMARILY IN SULPHATED MUCOPOLYSACCHARIDES (HERSBERGER, 1959). HIGH LEVELS OF CORTISONE OR ASCORBIC ACID DEFICIENCY PREVENT THE APPEARANCE OF STAINABLE, SULPHATED MUCOPOLYSACCHARIDE, DECREASE THE INCORPORATION OF LABELLED SULPHATE, AND PREVENT THE APPEARANCE OF RETICULAR AND COLLAGEN FIBERS.

WITH HIGH DOSES OF CORTISONE, 15 MG. PER DAY, GIVEN THREE DAYS BEFORE IMPLANTATION AND DAILY THEREAFTER TO RATS, THE IMPLANTATION OF COTTON SURGICAL SPONGE DID NOT LEAD TO FIBROPLASIA AND ANGIOPLASIA THAT OCCURS IN CONTROL ANIMALS (LATTES ET AL, 1953), UNLESS THE COTTON SPONGE IN THE CORTISONE ANIMALS WAS CONTAMINATED BY BACTERIA (LATTES ET AL, 1954). WHEN BACTERIA WERE INTENTIONALLY INJECTED IN COTTON SPONGE IMPLANTS, FIBROPLASIA AND ANGIOPLASIA OCCURRED IN SPITE OF ADEQUATE DOSAGE OF CORTISONE (LATTES ET AL, 1954).

LATTES ET AL (1954) PROPOSED THAT THE BACTERIA OR PRODUCTS BROUGHT ABOUT THE RELEASE OF ACID MUCOPOLYSACCHARIDES THAT WERE NECESSARY FOR THE FIBROPLASIA AND ANGIOPLASIA TO OCCUR. EVIDENCE TO SUPPORT THIS WAS OBTAINED WHEN THEY REPORTED THAT LOCAL INJECTION OF CARTILAGE EXTRACTS INTO THE COTTON SPONGES OVERCAME THE INHIBITORY EFFECT OF CORTISONE ON FIBROPLASIA (LATTES ET AL, 1956). SEVERAL OTHER AGENTS, INCLUDING TALC POWDER SUSPENSIONS AND MECHANICAL TRAUMA FAILED TO OVERCOME THE REPRESSIVE EFFECT OF CORTISONE.

THIS IS OF INTEREST IN RELATION TO THE CLINICAL OBSERVATIONS OF CLOSURE OF PERIODONTAL POCKETS. WHEN A POCKET WALL IS ACUTELY INFLAMED DUE TO BACTERIAL INVASION THE POCKET CLOSURE ATTAINED ON TREATMENT IS OFTEN MUCH MORE EXTENSIVE THAN THAT ATTAINED ON TREATMENT OF THE CHRONICALLY INVOLVED POCKET (RAMFGORD, 1953; ANDERSON, 1961). THE CLOSURE IS NOT JUST OF THAT PORTION OF THE POCKET THAT WAS FORMED DURING THE ACUTE PHASE, BUT INCLUDES PRE-EXISTING POCKET DEPTH THAT RESISTED PRIOR TREATMENT, INCLUDING MECHANICAL TRAUMA FROM

CURRETTAGE.

VARIOUS POLYSACCHARIDES, MOSTLY OF BACTERIAL ORIGIN, CAN INDUCE INFLAMMATORY CONDITIONS IN EXPERIMENTAL ANIMALS (MEIER ET AL, 1955; LANDY AND SHEAR, 1957). CARRAGEENIN, A SULPHATED POLYSACCHARIDE OF PLANT ORIGIN, INDUCES AN INFLAMMATORY CONDITION WHICH GRADUALLY IS TRANSFORMED INTO A FIBROUS GRANULOMA.

WHEN HYDROCORTISONE, 5 MG. DAILY, WAS ADMINISTERED FOLLOWING CARRAGEENIN IMPLANTATION IN GUINEA PIGS THE TISSUE RESPONSE WAS NOT AFFECTED QUALITATIVELY, WAS REDUCED QUANTITATIVELY, BUT WAS NOT BLOCKED (FISHER AND PARR, 1960). THIS STUDY DID NOT RELATE TO FIBROPLASIA IN WOUND HEALING OR IN OTHER GRANULOMATA. HOWES (1954) REPORTED THAT DOSAGE OF GLUCOCORTICOIDS IN REGARD TO TIME AND AMOUNT IS CRUCIAL IN DETERMINING WHETHER GRANULATIONS ARE DELAYED OR NOT, AND HOW LONG THE DELAY IS.

AN EXPERIMENT THUS WAS CARRIED OUT TO DETERMINE IF CARRAGEENIN GRANULOMATA WOULD FORM IN ANIMALS RECEIVING CORTISONE IN DOSAGE ADEQUATE TO DEPRESS FIBROPLASIA AND ANGIOPLASIA IN WOUNDS AND COTTON IMPLANTS.

METHOD

THE PROCEDURE OF LATTES ET AL (1954, 1956) WAS CLOSELY FOLLOWED -

WHITE RATS, 9 FEMALE AND 6 MALE, WERE USED.

CORTISONE ACETATE WAS INJECTED INTRAMUSCULARLY.

3 MALES 3 DAYS PRIOR TO IMPLANTATION RECEIVED 25 MG. CORTISONE PER DAY, AND AT IMPLANTATION AND DAILY THEREAFTER RECEIVED 17.5 MG.

3 FEMALES 3 DAYS PRIOR TO IMPLANTATION RECEIVED

17.5 MG. CORTISONE PER DAY, AND AT IMPLANTATION AND DAILY THEREAFTER RECEIVED 15 MG.

3 FEMALES AT IMPLANTATION RECEIVED ONE DOSE OF 15 MG.

3 MALES AND 3 FEMALES RECEIVED NO CORTISONE.

COTTON PELLETS No.3 DENTAL WERE USED. PELLETS WERE USED INSTEAD OF SPONGES BECAUSE THE RESPONSE TO PELLETS HAS BEEN EVALUATED IN REGARD TO WEIGHT AS WELL AS MORPHOLOGY BY MANY INVESTIGATORS INCLUDING HERSHBERGER ET AL (1959), MEIER ET AL (1940), MEYER ET AL (1953), DULIN (1955), CHRISTIAN AND WILLIAMSON (1958). THE COTTON SPONGES AS USED BY LATTES ET AL (1953, 1954, 1956) WERE EVALUATED ONLY MORPHOLOGICALLY. COTTON PELLETS WEIGHING BETWEEN 4-1/4 AND 5-3/4 MG. WERE SELECTED. TWO PELLETS WERE IMPLANTED SUBCUTANEOUSLY IN THE BACK OF EACH RAT VIA A MID LINE SAGITTAL INCISION. PELLETS ON THE LEFT SIDE WERE SOAKED IN STERILE 0.9% SALINE. PELLETS ON THE RIGHT SIDE WERE SOAKED IN STERILE DISTILLED WATER. EDWARDS AND DUNPHY (1957) NOTED THAT MINIMAL FIBROPLASIA RESULTED IN CAREFULLY IMPLANTED POLYVINYL ALCOHOL SPONGES WHEN THE IMPLANTS WERE SOAKED IN ISOTONIC SALINE, WHEREAS MARKED FIBROPLASIA WAS OBTAINED WITH WATER SOAKED IMPLANTS.

THE WOUND THAT WAS STUDIED WAS THE LINEAR INCISION THAT WAS MADE TO INTRODUCE THE COTTON PELLETS.

CARRAGEENIN, TYPE 21, WAS OBTAINED THROUGH THE COURTESY OF MR. L. STOLOFF, SEAPLANT CORPORATION. 5 C.C. OF 1% SOLUTION OF CARRAGEENIN IN 0.9% NA CL WAS INJECTED SUBCUTANEOUSLY IN THE LOWER ABDOMINAL REGION. THIS IS THE

TECHNIC USED BY FISHER AND PAAR (1960), JACKSON (1957), SLACK (1957), AND WILLIAMS (1957).

IMPLANTS AND INJECTIONS WERE MADE UNDER ETHER ANESTHESIA. ALL INCISIONS WERE CLOSED WITH A BLACK SILK SUTURE. IMPLANTS WERE LEFT TWO WEEKS. THIS IS LONG ENOUGH TO PERMIT CARRAGEENIN GRANULOMA FORMATION (FISHER AND PAAR, 1960), BENITZ AND HALL (1959) AND SHORT ENOUGH TO ENSURE CORTISONE SUPPRESSION OF FIBROPLASIA (HOWES, 1954). FIBER FORMATION OCCURS PART WAY INTO COTTON PELLETS AT THIS PERIOD OF TIME (HERSHBERGER ET AL, 1959).

PENICILLIN, BICILLIN 0.1 c.c. OF 30,000 UNITS PER C.C., WAS INTRAMUSCULARLY INJECTED DAILY IN ALL RATS TO ELIMINATE THE BACTERIAL EFFECT. LATTES ET AL (1954) NOTED THAT INFLAMMATION AND REPAIR WERE MINIMAL IN COTTON GAUZE IMPLANTS WHEN ANIMALS ON CORTISONE WERE PROTECTED WITH ANTIBIOTICS.

OBSERVATIONS

GROSS: ALL ANIMALS SURVIVED. THEY WERE SACRIFICED BY MEANS OF CHLOROFORM.

WEIGHT: CONTROL ANIMALS GAINED. MALES 1.5% GAIN (7 GMS. ON 467)
FEMALES 2.7% GAIN (6 GMS. ON 221)
CORTISONE ANIMALS LOST. MALES 31% LOSS (153 GMS. ON 493)
FEMALES 28% LOSS (75 GMS. ON 269)
CORTISONE SINGLE DOSE ANIMALS LOST 6.85% (19 GMS. ON 278)

WOUNDS: IN CONTROL RATS ALL INCISIONS WERE CLOSED.

IN CORTISONE RATS ONE INCISION WAS CLOSED, THE REMAINDER WERE OPEN.

IN SINGLE CORTISONE DOSE RATS ALL INCISIONS WERE CLOSED.

WOUNDS: (CONT'D.)

IN SINGLE CORTISONE DOSE RATS ALL INCISIONS WERE CLOSED.

PELLETS:

ALL BUT ONE (WATER SOAKED IN CORTISONE FEMALE) WERE LOCATED AWAY FROM THE INCISION.

IN CORTISONE TREATED ANIMALS PELLETS WERE WHITE.

IN CONTROL AND SINGLE INJECTED ANIMALS PELLETS WERE STREAKED WITH PINK.

CARRAGEENIN:

IN CONTROL ANIMALS THERE WAS NO EXTERNAL ENLARGEMENT.

THE UNDERSIDE OF THE SKIN WAS A LITTLE RAISED, BUT FLATTENED. THIS AREA WAS MORE FIRMLY ATTACHED TO THE SUBCUTANEOUS FASCIA THAN WAS THE SURROUNDING SKIN. RED VESSELS COULD BE SEEN IN THE GRANULOMA AREA AND FAIRLY LARGE VESSELS CAME TO THE GRANULOMA FROM THE INGUINAL REGION. THE GRANULOMA AREA WAS THICKER AND FIRMER THAN THE SURROUNDING SKIN.

THE CORTISONE ANIMALS WERE THE SAME AS THE CONTROLS FOR THE MALES AND SINGLE DOSE FEMALES. ONE CORTISONE FEMALE HAD A REDUCED AREA AND TWO CORTISONE FEMALES HAD NO DEFINITE GROSS INDICATION OF GRANULOMA.

WEIGHTS:

ALL PELLETS AND CARRAGEENIN-SKIN GRANULOMATA WERE WEIGHED. CARRAGEENIN-SKIN SPECIMENS WERE TRIMMED TO THE EDGE OF THE GRANULOMA AND MEASURED PRIOR TO WEIGHING. WEIGHTS FOR THESE ARE EXPRESSED IN MGMS. PER 1000 SQ. MM.

WEIGHTS: (CONT'D.)

AFTER WEIGHING SOME WERE SELECTED FOR PREPARATION FOR MICROSCOPIC EXAMINATION. THE REMAINDER WERE DRIED AT 60°C. AND WEIGHED AT 7 AND 11 DAYS. WEIGHTS WERE THE SAME AT BOTH DATES, INDICATING THE SPECIMENS HAD DRIED TO A CONSTANT WEIGHT.

PELLET WEIGHT IN MGS.

		<u>DRY</u>	<u>WET</u>	<u>RANGE</u>
CONTROL MALE	WATER	13.5	56.9	44-69
	SALINE	17	59.3	52.8-68
FEMALE	WATER	16	65.1	53-77
	SALINE	<u>14</u>	<u>66.2</u>	63.2-66.5
AVERAGE		<u>15.1</u>	<u>61.9</u>	
STORED PELLETS	WATER	5	85.5	
	SALINE	6	98.0	

		<u>DRY</u>	<u>WET</u>	<u>RANGE</u>
CORTISONE MALE	WATER	7.5	40.8	38-44.5
	SALINE	8.0	42.4	41.5-43.3
FEMALE	WATER	7.5	42.7	41-46
	SALINE	<u>8.0</u>	<u>42.7</u>	39.2-43.8
AVERAGE		<u>7.75</u>	<u>42.1</u>	
SINGLE DOSE FEMALE	WATER	11.5	55	48.5-60
	SALINE	<u>10.75</u>	<u>58.2</u>	54-64.3
AVERAGE		<u>11.1</u>	<u>56.6</u>	

CARRAGEENIN-SKIN WEIGHT IN MGS. PER 1000 SQ. MM.

		<u>WET</u>	<u>RANGE</u>	<u>DRY</u> (APPROX.)
CONTROL	MALE	4484	4106-4762	2188
	FEMALE	4539	4344-4637	1111
CORTISONE	MALE	4275	3672-4762	1212
	FEMALE	2143	1981-2344	800
I DOSE	FEMALE	3406	2874-3938	1378

MICROSCOPIC EXAMINATION:

CARNOY'S FIXATIVE WAS USED. WOUNDS WERE CROSS SECTIONED, PELLETS WERE SECTIONED AT THE CENTER, AND CARRAGEENIN GRANULOMATA WERE SECTIONED THROUGH THE GREATEST BULK. STAINING METHODS:- H.E. VAN GIESON, PERMANGANATE OXIDATION-ALCIAN BLUE, PAS, SILVER RETICULIN, AZURE A-CITRATE-PHOSPHATE-ALCOHOL FOR ALL, AND FOR GRANULOMATA ALSO USED SULPHATION-AZURE A AND SULPHATION-ALCIAN BLUE.

OBSERVATIONS:

WOUNDS: IN THE CONTROLS, THE INCISIONS WERE COMPLETELY HEALED. COLLAGEN FIBERS WERE NOT AS LARGE AS THOSE OF THE SURROUNDING DERMIS. THERE WAS A GREATER CONCENTRATION OF ACID MUCOPOLYSACCHARIDES, AS JUDGED BY THE OXIDATION-ALCIAN BLUE METHOD, AND MORE CELLS AT THE WOUND THAN IN THE REST OF THE PAPILLARY LAYER OF THE DERMIS. THE CORTISONE TREATED ANIMAL WITH THE CLOSED WOUND SHOWED EPITHELIALIZATION WAS COMPLETE, BUT EXCEPT FOR IMMEDIATELY UNDER THE EPITHELIUM THERE WAS NO EVIDENCE OF CONNECTIVE TISSUE FORMATION. IN AN OPEN WOUND CONTAINING BACTERIA THERE WAS A LOCALIZED INVASION BY BACTERIA. TISSUE ADJACENT TO THE BACTERIA WAS OXIDATION-ALCIAN BLUE POSITIVE.

PELLETS:

CONTROL: COLLAGEN FIBERS WERE IRREGULARLY ARRANGED, AND EXTENDED APPROXIMATELY HALF WAY INTO THE PELLETS. AROUND FIBERS WAS OXIDATION-ALCIAN BLUE POSITIVE MATERIAL. THERE WERE FOREIGN BODY GIANT CELLS NEXT TO THE COTTON FIBERS. THESE CELLS CONTAINED RIBONUCLEIC ACID IN THE CYTOPLASM AND NUCLEOLI, AND THE NUMEROUS FIBROBLASTS ALSO CONTAINED RNA IN THE CYTOPLASM. THERE WERE NUMEROUS BLOOD VESSELS PRESENT. EXCEPT IN THE CAPSULE THERE WAS NO EVIDENCE OF METACHROMATIC GRANULES WITHIN CELLS, EVEN AFTER SULPHATION.

CORTISONE: IN ALL ANIMALS THE PELLETS CONTAINED NO CELLS; NO INTERCELLULAR MATERIAL, FIBRILLAR OR AMORPHOUS; AND NO BLOOD VESSELS. THERE WAS JUST SOME DEBRIS IN THE PELLETS, AND A NARROW CAPSULE AROUND PART OF SOME OF THE PELLETS.

SINGLE INJECTION CORTISONE: FIBERS AND RELATED SUBSTANCES WERE PRESENT SIMILAR TO THE CONTROL ANIMALS, BUT NOT AS FAR INTO THE PELLETS.

CARRAGEENIN GRANULOMATA:

CONTROL AND CORTISONE ANIMAL GRANULOMATA BOTH CONTAINED MANY MACROPHAGES WITH INTERCELLULAR, CARRAGEENIN, BLOOD VESSELS WITH COLLAGEN FIBER SUPPORT, RETICULAR FIBERS, AND SOME CURLY STRANDS OF NON ORIENTATED COLLAGEN FIBERS. IN THE MALES THERE WAS MORE ADIPOSE TYPE TISSUE AND THE GRANULOMA WAS LARGER IN CROSS SECTION AREA. IN THE CORTISONE INJECTED ANIMALS THERE WERE MORE COLLAGEN FIBERS IN THE CARRAGEENIN GRANULOMA THAN IN THE CONTROL ANIMALS. IN THE FEMALE CORTISONE TREATED ANIMALS THE GRANULOMA WAS SMALLER AND THERE WAS MORE FREE CARRAGEENIN. CARRAGEENIN GRANULOMATA IN THE FEMALE ANIMALS

WITH THE SINGLE CORTISONE INJECTION WERE SIMILAR TO THE CONTROL FEMALES.

IN THE CONTROL AND CORTISONE ANIMALS THERE WAS EVIDENCE OF EXTRACELLULAR ACID MUCOPOLYSACCHARIDES (OXIDATION-ALCIAN BLUE METHOD). THE INTENSITY WAS NOT AS GREAT AS IN THE PELLETS AND WOUNDS HOWEVER. SINCE AN ALCOHOL FIXATIVE WAS USED THE CARRAGEENIN WAS RETAINED AND STAINED INTENSELY WITH ALCIAN BLUE, AZURE A AND PAS.

DISCUSSION:

MARKED WEIGHT LOSS OF APPROXIMATELY 30% INDICATES THAT CORTISONE HAD A SEVERE EFFECT ON THE METABOLISM OF THE RATS.

EXAMINATION OF PELLETS FROM ASPECTS OF BOTH WEIGHT AND MORPHOLOGY, AND WOUNDS GROSSLY AND MICROSCOPICALLY, INDICATES THAT CONNECTIVE TISSUE FORMATION WAS APPARENTLY TOTALLY INHIBITED. THIS AGREES WITH LATTES ET AL (1953). THE GROWTH OF CONNECTIVE TISSUE IN THE CONTROLS ALSO AGREES WITH THE OBSERVATIONS OF LATTES ET AL (1953), AND OTHERS. GROWTH IN THE COTTON PELLET WAS THE SAME AS IN THE COTTON SPONGE. THE GROWTH OF CONNECTIVE TISSUE INTO THE COTTON PELLET HALF WAY RATHER THAN ON THE OUTSIDE AGREES WITH THE OBSERVATIONS OF HERSHBERGER ET AL (1959). EPITHELIALIZATION OF THE WOUND IN SPITE OF CORTISONE AGREES WITH HOWES (1954). THE SLIGHT CAPSULE FORMATION PART WAY AROUND SOME OF THE PELLETS IN THE CORTISONE TREATED ANIMALS WAS NOT NOTED BY LATTES ET AL (1954) WITH HIS SPONGES. IN THIS CONNECTION GOULD (1961) REPORTED THAT WHEN GUINEA PIGS WERE DEPRIVED OF ASCORBIC ACID A CAPSULE FORMED AROUND POLYVINYL ALCOHOL SPONGE IMPLANTS, BUT COLLAGEN FIBERS DID NOT FORM WITHIN THE SPONGE

OR IN WOUNDS AS THEY DID IN THESE SITES IN CONTROL ANIMALS. HE SUGGESTS THAT THERE IS A DIFFERENCE BETWEEN THE COLLAGEN OF THE CAPSULE AND THE COLLAGEN FOUND WITHIN THE IMPLANTS AND IN REPAIR.

THE SIMILAR WEIGHTS OF SALINE SOAKED AND WATER SOAKED PELLETS IN CONTROL ANIMALS INDICATE THAT THE COTTON PELLETS ARE IRRITATING ENOUGH IN THEMSELVES TO INITIATE THE GRANULOMA FORMATION, OR THAT THE IMPLANTATION TECHNIC USED CAUSED ENOUGH MECHANICAL TRAUMA TO SET OFF THE REACTION. COTTON IS MORE IRRITATING THAN POLYVINYL ALCOHOL AS JUDGED BY THE PRESENCE OF FOREIGN BODY GIANT CELLS. TURPENTINE SOAKED PELLETS WERE REPORTED TO INCREASE COTTON PELLET GRANULOMA WEIGHT THREEFOLD IN A SIX DAY IMPLANT PERIOD (CHRISTIAN AND WILLIAMSON, 1958).

EVEN THE ONE LOW WET WEIGHT COTTON PELLET IN THE CONTROL ANIMAL WAS FOUND ON MICROSCOPIC EXAMINATION TO CONTAIN THE USUAL AMOUNT OF COLLAGEN FIBERS. THE INCREASE IN DRY WEIGHT OF PELLETS OF CORTISONE TREATED ANIMALS OVER STORED AND DRIED PELLETS WAS NOT DUE TO TISSUE FORMATION BUT APPARENTLY DUE TO DEBRIS. THIS DEBRIS IS APPARENTLY DERIVED FROM REMNANTS OF LEUCOCYTES (HERSHBERGER ET AL, 1959). CORTISONE DOES NOT INHIBIT THE EARLY INFLAMMATORY RESPONSE (LATTES ET AL, 1953). IN SPITE OF THIS NEGLIGIBLE EFFECT OF CORTISONE ON THE EARLY INFLAMMATORY RESPONSE, AND IN SPITE OF THE OBSERVATION THAT COLLAGEN FORMATION DOES NOT BEGIN IN WOUNDS UNTIL THE 3RD OR 4TH DAY (HOWES, 1954); THE SINGLE INJECTION OF CORTISONE IN FEMALES AT THE TIME OF IMPLANTATION OF THE COTTON PELLETS

AND CARRAGEENIN DID APPEAR TO REDUCE THE FIBROPLASIA, IN THAT THE WEIGHTS OF THE PELLETS AND CARRAGEENIN GRANULOMATA WERE BETWEEN THOSE OF THE CONTROL AND THE TOTAL PERIOD CORTISONE FEMALES. TOTAL WEIGHT CHANGE OF THESE SINGLE DOSE ANIMALS ALSO WAS BETWEEN FULL DOSE AND CONTROL ANIMALS.

THE ABSENCE OF METACHROMATIC GRANULES AFTER SULPHATION OF FIBROBLASTS IN THE COTTON PELLETS DIFFERS FROM THE OBSERVATIONS OF WINDRUM (1958) CONCERNING SUCH GRANULES IN FIBROBLASTS FROM SOME OTHER SITES.

THE CARRAGEENIN GRANULOMATA MACROSCOPICALLY AND MICROSCOPICALLY WERE SIMILAR TO THOSE DESCRIBED BY BENITZ AND HALL (1959), AND FISHER AND PAAR (1960). CARRAGEENIN IN THE RAT STIMULATES A MORE VIOLENT, ACUTE INFLAMMATION, MORE HISTIOCYTES, AND LESS COLLAGEN FIBER FORMATION THAN IN THE GUINEA PIG, BUT THE SEQUENCE OF EVENTS IS SIMILAR.

IN SPITE OF CORTISONE ADEQUATE ENOUGH TO CAUSE 30% WEIGHT LOSS, AND TOTAL INHIBITION OF CONNECTIVE TISSUE GROWTH IN WOUNDS AND IN COTTON PELLETS, CARRAGEENIN STIMULATED IN THE SAME MALE ANIMALS AS MUCH GRANULOMA, AND IDENTICALLY COMPOSED GRANULOMATA, AS THOSE FOUND IN THE CONTROL ANIMALS. COLLAGEN FIBER FORMATION WAS EVEN GREATER IN THE CORTISONE ANIMALS. ACID MUCOPOLYSACCHARIDE ACCUMULATION, RETICULAR FIBER FORMATION, HISTIOCYTES, AND ADIPOSE TISSUE FORMATION WERE SIMILAR IN MALE CORTISONE AND CONTROL ANIMALS.

IN THE CORTISONE FEMALES THE REACTION WAS PRESENT AND SIMILAR QUALITATIVELY TO THE CONTROLS BUT LESS QUANTITATIVELY.

A POSSIBLE REASON MAY BE IN TECHNICAL ERROR IN THAT THE CORTISONE FEMALES WERE INJECTED FIRST WITH THE CARRAGEENIN, AND SOME MAY HAVE BEEN DEPOSITED IN THE WRONG PLACE. THERE MAY, HOWEVER, BE AN ACTUAL SEX DIFFERENCE. ANOTHER POSSIBILITY IS IN REGARD TO THE DOSE. DURING THE GRANULOMA FORMATION PERIOD THE FEMALES RECEIVED MORE CORTISONE PER KG. THAN DID THE MALES, 269 GM. AVERAGE FEMALES RECEIVED 15 MG. AND 493 GM. MALES RECEIVED 17.5 MG. MALES, HOWEVER, SHOWED EVEN GREATER PERCENTAGE WEIGHT LOSS ON CORTISONE.

THE OBSERVATIONS SUPPORT THE HYPOTHESIS THAT SULPHATED POLYSACCHARIDE FORMATION IS BLOCKED BY CORTISONE, AND THAT SULPHATED POLYSACCHARIDE IS NECESSARY FOR FIBER FORMATION, ENDOTHELIAL PROLIFERATION, AND FOREIGN BODY GIANT CELL FORMATION. IT APPEARS THAT INTRACELLULARLY LOCATED, (IN MACROPHAGES), PLANT DERIVED SULPHATED POLYSACCHARIDE CAN OVERCOME THE REPRESSIVE EFFECT OF CORTISONE ON CONNECTIVE TISSUE FORMATION. THESE RESULTS ARE ESSENTIALLY IN ACCORD WITH WHAT MIGHT BE EXPECTED IN LIGHT OF THE FINDINGS OF LATTES ET AL (1954, 1956).

FISHER AND PAAR (1960) FURTHER POSTULATED THE INTRACELLULAR CARRAGEENIN ACTED TO THE EXTENT THAT EXTRACELLULAR ACID MUCOPOLYSACCHARIDE WAS NOT FORMED IN THE CARRAGEENIN GRANULOMA. THEY COULD NOT FIND ANY EVIDENCE OF EXTRACELLULAR ACID MUCOPOLYSACCHARIDE WITH THE USUAL STAINS FOR SUCH MATERIAL. WILLIAMS (1957), HOWEVER, REPORTS PRESENCE OF STAINABLE ACID MUCOPOLYSACCHARIDE IN CARRAGEENIN GRANULOMA. SULPHATE AND HEXOSE DETERMINATION OF CARRAGEENIN GRANULOMA AFTER S³⁵ INJECTION

SUGGESTS EARLY RAPID SYNTHESIS OF SULPHATED POLYSACCHARIDE IN THESE GRANULOMATA (SLACK, 1957). IN THE PRESENT INVESTIGATION AS WITH FISHER AND PAAR (1960), NO EXTRA-CELLULAR MATERIAL OTHER THAN CARRAGEENIN STAINED METACHROMATICALLY. OXIDATION-ALCIAN BLUE POSITIVE MATERIAL WAS FOUND THOUGH. THIS TECHNIC WAS FOUND TO GIVE THE MOST INTENSE STAINING OF VARIOUS METHODS IN THE STUDY OF YOUNG SKIN, WOUNDS, AND IN GRANULOMA OF POLYVINYL ALCOHOL SPONGES AND COTTON PELLETS (ANDERSON, 1959).

WILLIAMS (1957), BENITZ AND HALL (1959), AND FISHER AND PAAR (1960) SUGGEST THAT THE HIGH DEGREE OF SULPHATION OF CARRAGEENIN (23%) IS PROBABLY AN IMPORTANT FACTOR IN EVOKING THE GRANULOMATOUS REACTION. METHYL CELLULOSE, POLYVINYL ALCOHOL, DEXTRAN, PECTIC ACID (WILLIAMS, 1957) AND AGAR (MEIER ET AL, 1955) FAILED TO GIVE A RESULT SIMILAR TO CARRAGEENIN.

CONCLUSION AND SUMMARY:

CARRAGEENIN, A SULPHATED POLYSACCHARIDE, INDUCED A CHARACTERISTIC GRANULOMA FORMATION IN RATS (ESPECIALLY MALES) IN SPITE OF CORTISONE IN AMOUNTS ADEQUATE TO PREVENT WOUND HEALING AND COTTON PELLET GRANULOMA FORMATION IN THE SAME ANIMALS.

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APPENDIX B

Progress Report 1961-1962 to Associate Committee on Dental Research

National Research Council

Grantee: Charles M. Dowse.

Project No: DA-81.

Project Title: Metabolism of Periosteum.

It proved impossible during the past year owing to sporadic disruptions due to continued building operations to carry out the work with glucose C¹⁴ outlined in last years application. This phase of the project inso far as point (a) the assessment of the activities of the TCA cycle and the hexosemonophosphate pathway in the tissue is concerned, will be carried forward during the coming year.

Certain results obtained using iodoacetate, however, suggest, in a gross way, that the HMP pathway is, at least potentially, present in the tissue.

Glycolysis in calvaria incubated in Krebs phosphate medium is almost completely inhibited by iodoacetate at a concentration of 10^{-3} M under both aerobic and anaerobic conditions. The addition of pyruvate produced a marked increase in the quantity of lactic acid accumulating in the presence of iodoacetate but had no effect when the latter was absent. (Table 1.)

The results are consistent with the operation, under the experimental conditions employed, of the pentose phosphate pathway, if, as has been shown to be the case for the cornea by Kinoshita (1957) as well as for the retina by Kinoshita and Futterman (1959) and Cohen and Noell (1960), pyruvate stimulates the metabolism of glucose via this route by accepting electrons from TPNH. Iodoacetate and anaerobiosis also increase the

activity of the pentose phosphate pathway, so conclusions as to its functioning in the tissue under normal conditions and as to its relative importance versus the TCA cycle in carbon oxidation cannot be reached until the work with glucose -C¹⁴ has been performed.

In earlier work, as reported last year, parathyroid hormone, added in vitro to the incubation medium at a level of 5 i.u. per ml., had been found to increase the net production of lactic acid by calvaria. It was postulated that this effect could be the result of an increased intracellular concentration of inorganic phosphate in response to the hormone. (Terepka et al. 1960). Glycolysis in the Ehrlich ascites cell has been shown to be independent of glucose concentration above 4 mg% (Kun et al 1951) while being very sensitive to changes in the concentration of inorganic phosphate in the medium (Wu and Racker 1959). The effect of adding Parathormone in vitro on glycolysis in the ascites cell was therefore investigated.

Ascitic fluid from CF-1 mice was collected directly into 4 volumes of cold Tris buffered medium and the cells washed twice. Erythrocytes contaminating the preparations were removed by slow centrifugation (McKee et al 1953). Incubations were carried out at 37°C in the same medium, having the following composition. Tris buffer pH 7.4 3×10^{-2} M: Na₂HPO₄ pH 7.4 4×10^{-3} M: NaCl 14×10^{-3} M: KCl 4×10^{-3} M: MgSO₄ 1.5×10^{-3} M.

Parathormone (Lilly) was added in the cold at a level of 1 i.u. per ml. After 2 minutes the flasks were transferred to a 37°C bath and after a further 3 minutes to allow for temperature equilibration, glucose was added. Samples were withdrawn at varying time intervals, deproteinised with Ba(OH)₂/ZnSO₄ and analysed for glucose and lactic acid by the glucose oxidase and lactic dehydrogenase methods respectively.

The results obtained so far are given in Tables 2 and 3 and indicate a considerable effect of the extract at this concentration on the initial rate of glucose uptake by the ascites cell. No significant effect has as yet been observed upon lactate production.

If further work corroborates the above positive finding and if an explanation can be found for the difference in effect observed vis a vis bone, further study of the metabolic effects of parathyroid hormone in vitro could more usefully be carried on using such an homogeneous preparation.

During the year, a considerable amount of time was devoted to the establishment of satisfactory procedures for cell and tissue culture as it was considered that some aspects of the problems of interest could be more readily attacked in this way. The Hela cell was used as an initial test cell for this purpose and when this could be handled satisfactorily the culture of bone was attempted.

Parietal bone from new born rats was cultured using essentially the same media and methods as Goldhaber (1958). The bone preparations were mounted on a coverslip in a clot composed of 2 parts chick plasma to 1 part chick embryo extract. The coverslips were placed in Leighton tubes containing medium composed of chick embryo extract 20% and horse serum 20% in Grey's balanced salt solution. Penicillin and streptomycin were present at 20 units per ml. The tubes were rotated in a roller drum at 1/5th r.p.m.

Adequate maintenance of structure and cell morphology was obtained when cultures are incubated for periods of up to seven days in air alone. The rapid resorption noted by Goldhaber in mouse calvarium under roller

drum conditions in air has not yet been observed. The difference may be due to the different species employed, It may also be due to the fact that so far we have been using only the parietal bone while Goldhaber utilised the whole calvarium and noted that resorption began first in the frontal bone.

With further development of tissue culture technique in our hands it is hoped that bone cultures may be utilised to carry out metabolic studies of the type already performed on calvaria in inorganic media. Also, bone in culture could well be a more suitable preparation to continue a study of the metabolic effects of PTH on this tissue as it is reasonable to expect that addition of PTH will be more effective under culture conditions. Some correlation can also be attempted between the morphological and metabolic changes resulting from alterations in the environment.

TABLE 1.

Additions	Lactic acid production by calvaria. (μ moles/ gm. wet wt./ hr.)	
	<u>Aerobic</u>	<u>Anaerobic</u>
Nil	13.0	24.0
Pyruvate	13.0	-
Iodoacetate	0.58	0.59
Pyruvate: iodoacetate	1.91	2.41
90 minute incubation: Krebs Ringer phosphate		
Glucose $5.5 \times 10^{-3} M$: Iodoacetate $1 \times 10^{-3} M$: Pyruvate $1 \times 10^{-2} M$.		

TABLE 2

Time period (mins.) Cumulative glucose uptake by ascites cells ($\mu\text{g. glucose per } 10^7 \text{ Cells}$)

	Control	PTE Added (1 i.u. per ml)
0-15	126	196
30	269	303
45	333	363
60	389	403

Glucose concentration 1000 $\mu\text{g/ml.}$ Cell concentration 1×10^7 cells/ml.

TABLE 3

Time period (mins.) Cumulative glucose uptake by ascites cells ($\mu\text{g. glucose per } 10^7 \text{ cells}$)

	Control	PTE added (1 i.u. per ml)
0-2	4.5	47.0
4	17.3	71.0
6	35.0	77.0
8	66.3	90.0
10	83.1	100.0

Glucose concentration 130 $\mu\text{g/ml.}$ Cell concentration 1×10^7 cells/ml.

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A series of controls were set up as follows:

1. Ten rats were used as absolute controls. The anterior pituitary was removed in each case and five were sacrificed after four weeks and five after twelve weeks. They were not given any hormone replacement therapy and their diet and conditions were maintained as before, except that they were kept in a room with a minimum temperature of 74°F.
2. Five rats were hypophysectomized and then two weeks later given 1/3 of a grain of thyrolid daily for 14 days, after this, they were sacrificed.

APPENDIX C

An Investigation of the Effect of Hypophysectomy and Hormone Replacement Therapy on the Oral Tissues of Female Rats

Project DA 87

PROGRESS REPORT

John Findlay

Methods: The anterior pituitary glands of the rats were removed by dissecting down to the base of the skull, and after retracting the oesophagus and trachea, opening into the pituitary fossa by drilling a hole in the skull just anterior to the basion, and by the use of negative pressure on a suction pump the anterior pituitary lobe was removed. A piece of gingival tissue was removed, at this time, and again before and after replacement therapy.

A series of controls were set up as follows:

1. Ten rats were used as absolute controls. The anterior pituitary was removed in each case and five were sacrificed after four weeks and five after twelve weeks. They were not given any hormone replacement therapy and their diet and conditions were maintained as before, except that they were kept in a room with a minimum temperature of 74°F.
2. Five rats were hypophysectomized and then two weeks later given 1/3 of a grain of thyroid daily for 14 days, after this, they were sacrificed.

3. Five rats were hypophysectomized and two weeks later given 5 micrograms of hydrocortisone daily for 14 days and then sacrificed.
4. Five rats were hypophysectomized and two weeks later given 20 milligrams of oestradiol-3-Benzate daily for 14 days, then sacrificed.
5. Five rats were hypophysectomized and two weeks later given 5 mgms. testosterone propionate.
6. Controls 2-5 were repeated without hypophysectomy being performed on the rats.
7. Five rats were hypophysectomized and seven days later were given 5 micrograms of cortisone daily for 30 days and 20 milligrams of oestrogen daily for 16 days. The oestrogen was started 20 days after the hypophysectomy.
8. Five rats were hypophysectomized and seven days later given 5 micrograms of cortisone daily for 30 days. At present a second series with the appropriate variations and controls has been set up.

In every case a gingival papilla was removed for microscopical examination at the time of hypophysectomy, before hormone replacement treatment, and again if there was any addition to hormone treatment. After the animals were sacrificed the remains of the pituitary were removed and examined to ensure that the anterior lobe was removed entirely. The mandible of each rat was removed and sectioned, and also the pertinent

organs and glands for confirmation and comparison. (The vagina, uterus, ovaries and adrenals.)

Results: The microscopic picture of the gingival tissue of the absolute controls was one of marked decrease of activity in both the basal cell layer and the prickle cell layer, the keratinised layer was very thin. The connective tissue was quite inactive. The periodontal fibres were more widely spaced and looser than normal, the membrane was wider than normal due to resorption of alveolar bone and the insertion of the fibres was into a poorly calcified osteoid type of tissue. The vascularity of the membrane was reduced. The group of rats which was left for the 12 weeks period showed the above picture in a more exaggerated fashion.

The control group which was given thyroid exhibited a similar microscopic picture to the above controls, in other words, low activity in the epithelium and connective tissue, replacement of marrow by fibrous tissue, widening of the periodontal membrane and bone resorption, the one difference being that in three of the five cases there was extensive haemorrhage into the membrane. No difference was noted in the pre- and post-operative examinations for glycogen and mucopolysaccharides in the oral tissue.

The control rats which were given cortisone exhibited a microscopical picture of thickened keratin and a basal cell layer and prickle cell layer with only slight activity but somewhat more than in the absolute controls. The connective

tissue was not active. The blood vessels were more numerous. The periodontal membrane was more vascular, but there were still signs of bone resorption. An interesting point noted in the teeth of persistent growth was that in every case there had been a disturbance in amelogenesis caused by haemorrhage which appeared to be into the area between the enamel organ and the alveolus. In every case where cortisone had been administered, there was an increase of glycogen in both the epithelium and connective tissue. This is consistent with the belief that cortisone counteracts the effect of hyaluronidase and allows a greater accumulation of glycogen in the tissues during the administration of cortisone.

The control rats which were given oestrogen showed a quite different microscopical picture. The keratin was thickened and there was marked activity in both the basal cell layer and the prickle cell layer of the epithelium. The connective tissue was active. The periodontal membrane was much more compact, and there were no signs of marked osteoclastic activity. Again an interesting phenomenon appeared in the enamel organ, this being an apparent increase of ameloclastic activity accompanied by the formation of poorly calcified enamel. Hertwigs' epithelial sheath exhibited signs of hyperactivity, the tip of the sheath being obviously overproliferated.

The control rats which were given testosterone exhibited the most normal microscopic picture of all. The keratin was thick and compact, both the prickle cell layer and the basal

cell layer were active and the connective tissue also was active and coarser than in any of the other controls. The periodontal membrane appeared to be normal, and so also did the enamel organ, and dentin papilla. There were no signs of excessive proliferation in Hertwigs' Sheath.

The rats which were hypophysectomied and given cortisone and oestrogen exhibited the following microscopical picture:

1. After cortisone had been given alone, the keratin was thick, but the basal cell layer and the prickle cell layer were inactive. The connective tissue was low in activity.
2. After oestrogen was added the prickle cell layer and the basal cell layer were very active and so also was the connective tissue. The keratin in these cases appeared excessive. The periodontal membrane was compact but there were still signs of osteoclastic activity. There were signs of haemorrhage into the periodontal membrane and also into the dentin papilla and in every case there was a disturbance in amelogenesis.

The group of control rats which was not hypophysectomied, but given the various hormones exhibited the following picture:

1. Those rats given thyroid exhibited no significant change.
2. Those rats given cortisone exhibited little change apart from a thickening of the keratin and an increase

in glycogen content of the tissue. There was a disturbance in amelogenesis.

3. Those given oestrogen showed a thickened keratinised layer, and a marked increase in activity of the basal cell layer and also of the prickle cell layer. A disturbance in amelogenesis occurred in every case.

4. Those rats given testosterone had a thickened epithelial covering. The keratin was thick and compact. The basal cell layer was active and so also was the prickle cell layer. There was no disturbance in amelogenesis.

This present study has not progressed sufficiently to allow any final conclusions to be drawn. It is apparent that hypophysectomy does cause a degenerative change in the periodontium, and that certain hormones have the ability to counteract these changes.

THE STORAGE MEDIUM FOR A FUNCTIONAL TOOTH BANK

Project DA-88

In the first phase, the proposed methods of determining viability following storage were evaluated with freshly extracted third molars from 35-day Syrian hamsters, the same teeth which would be used in latter culture studies. Third molars were sectioned and stained with hematoxylin and eosin as controls. Other teeth were incubated at 37°C in a nutrient media containing trypan blue and were examined histologically for uptake of the dye. A limited number of cells near the apex of the tooth exhibited granules within the cytoplasm. It was impossible to be certain whether the response was that of diffusion or active phagocytosis. Immediate transplants were introduced into the axillary space and recovered after thirty days. Clinical observation showed no acute inflammatory response around the explant yet there was no gross evidence of root development. Histologically the teeth were seen to be generally surrounded by chronic inflammatory cells including lymphocytes, macrophages and giant cells. This foreign body reaction was occasionally seen to extend into the pulp chamber. The specimens in which the response was less pronounced showed normal pulp architecture; the odontoblast layer and the young fibroblastic pulp tissue being well preserved. A layer of osteodentin was frequently deposited at the apex and along the apical portion of the pulp canal. This was the only positive evidence of root maturation. Histo-

chemical studies were first attempted on frozen sections of human pulp obtained by fracturing young teeth and removing the pulp. Attempts to repeat these procedures on hamster molars failed due to the minute quantity of tissue. The histochemical staining of hamster pulp was not considered worth continued experimental study since its value as an indicator of viability would be questionable even if the procedure became feasible.

The second phase of the study involved the incubation of developing third molars in culture media of varying complexity. A base line was established by initially culturing the teeth in a balanced salt solution. (Hanks' solution was substituted for Earle's to eliminate the necessity of equilibrating with 5% gaseous CO₂, a procedure which is only of questionable value over buffering with phosphates and carbonate). Into one half the culture dishes, a minute amount of trypan blue was introduced. The teeth were incubated at 37°C for four days.

The teeth were removed from culture and one half were introduced into the axilla. The remainder were either examined for trypan blue or were stained with hematoxylin and eosin. The H and E sections were characterized by a pulp undergoing early necrosis. The pulpal architecture was well seen, but cellular changes were those of increased granulation, shrinkage, and loss of staining power particularly in the odontoblast layer. The sections

incubated with trypan blue showed no granules, suggesting that active phagocytosis may have accounted for their presence in immediate transplants. The axillary explants were again treated as foreign bodies. Several pulps were completely invaded by inflammatory cells, while others showed replacement of necrotic areas with fibrous connective tissue. It was concluded that the balanced salt solution alone was not capable of maintaining the dental pulp "in vitro".

The next study evaluated the preservation of potential transplants in Hanks' salt solution with 10% hamster serum. The teeth were incubated and evaluated as described above. The pulp preservation as shown by H and E was superior to that in the first experiment. There was less shrinkage of the odontoblast layer and the cellular detail was more distinct. The cytoplasm of the odontoblast was granular in the deeper portions of the pulp but showed less vacuolation. The fibrous pulp tissue was well maintained. The odontoblast layer appeared more sensitive to the trauma of tissue culture than the remaining pulp tissue. No evidence of trypan blue could be demonstrated. The axillary transplants were plagued by invasion of inflammatory cells. The characteristic finding was a fibrous connective tissue replacement of the pulp with no root maturation. The preservation of the pulp in the serum medium was seen to exceed that of the salt solution alone with ordinary histologic procedures.

The attempt to evaluate this improvement by continued growth and cellular activity failed.

Additional investigations will be undertaken to evaluate potential transplants in the other media outlined in the proposal. Also attempts will be made to reduce the inflammatory response to axillary tooth transplants by treating the animals with cortisone.

APPENDIX E

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH OF THE NATIONAL RESEARCH COUNCIL

December, 1961

Grantee: A. M. Hunt

Project: DA-82 Amount Awarded: \$6860. (1961-62)

Subject: Measurement of Strontium⁹⁰ and Carbon ¹⁴ in Primary
Teeth of Canadian Children.

Introduction

The project was outlined in detail in the 1960 progress report to the Associate Committee on Dental Research. The present report deals only with work completed in 1961.

Summary of Work

Work in 1961 was confined to three phases: the perfection of techniques for the separation of Strontium⁹⁰ and Yttrium⁹⁰, the analysis of cattle teeth, and the collection of samples.

Separation of Strontium⁹⁰ and Yttrium⁹⁰

Since the method of Bryant, Morgan and Spicer¹ gave low and unpredictable yields of carrier strontium, each step of the procedure was examined carefully, in collaboration with Dr. Ralph Burgess, to improve this yield. Losses were largely due to the difficulty encountered in decanting supernatants and transferring residues, the co-precipitation of Sr as SrCrO_4 during the barium chromate scavenge and failure to cool the solutions following the iron scavenge. Adjustment of these procedures improved the SrCo_4 yield by 20 to 25 per cent. This increased efficiency also made possible the reduction in weight of the ashed samples from 5.0 to 2.5 grams. In addition, the method was streamlined so that up to 30 samples could be prepared simultaneously.

Analysis of Cattle Teeth and Bone

One of the stated aims of the project was to give information about possible differences in the uptake of Sr^{90} by bone, dentin and enamel. Since human bone was not available, samples of bone, enamel, and dentin were obtained from 7 calves and cows, 3 months to 2 years of age. The specimens were obtained from Powassan and Lindsay and had been born and reared within 3 miles of these towns. Strontium⁹⁰ samples were obtained from the specimens and counted on a low background counter which had been standardized with $\text{Sr}^{90}\text{CO}_3$ samples from the Health and Safety Laboratories of the United States Atomic Energy Commission. Activities were expressed as $\mu\mu$ curies of Sr^{90} per gram of calcium. The results are summarized below.

1. There was no appreciable difference in activity in the samples of bone and dentin obtained from the same animal.
2. There was a significant difference in the uptake of Sr^{90} by enamel and dentin. Enamel from each of the seven animals contained considerably less Sr^{90} than did the dentin. The average difference was 16.9 per cent and the range of differences was 12.3 to 20.7 per cent.
3. The range of activities for 1959 samples was 35.5 to 43.7 $\mu\mu\text{c Sr}^{90}/\text{gm Ca}$ while the range of activities for 1961 samples was considerably lower, 16.5 to 21.5 $\mu\mu\text{c Sr}^{90}/\text{gm Ca}$.

The finding that the measured amount of Sr^{90} in enamel is approximately 17 per cent less than that in dentin is not explainable by the fact that the bulk of enamel forms before dentin since both tissues were formed towards the end of the nuclear test ban. At this period, it can be presumed that

similar amounts of Sr^{90} were available to both enamel and dentin. However, Posner and Likens² have shown that the discrimination against Sr relative to Ca increased with an increase in crystal size and perfection in synthetic hydroxyapatites of varying sizes. They³ also showed that the enamel of rats injected with Sr^{89} and Ca^{45} incorporated as much as 24 per cent less Sr^{89} than did bone and dentin. This discrimination by the larger crystals of enamel seems to point to a crystal surface adsorption effect. Since the exfoliated incisor crowns of primary teeth have a proportionately large enamel/dentin ratio, it seems advisable to continue with the separation of enamel and dentin fractions with the expectation that the dentin fractions will give a better correlation with bone.

The very high activities found in cattle teeth in 1959 (35.7 to 43.7 $\mu\mu\text{c}$ $\text{Sr}^{90}/\text{gm Ca}$) are probably higher than those in human teeth from the same year by a factor of ten. In addition, some consideration should be given to a study of the incidence of bone tumours in cattle since these activities represent approximately half of the maximum permissible concentration in bone beyond which some bone tumours are expected to be produced. Such a study could lead to an adjustment of the estimated maximum permissible concentration in bone.

Collection of Tooth Samples

Arrangements have been made to monitor the entire tooth sample from the Montreal Baby Tooth Survey. Sufficient teeth were received on December 12th to measure samples for the years 1950 to 1955. The Department of Dental Public Health in

Toronto and Vancouver are collecting samples.

Pertinent Studies Published in 1961

Reiss⁴ has recently published an excellent paper on Sr⁹⁰ absorption by primary teeth of children from St. Louis, Mo. Useful information has been recorded regarding the relative uptake of Sr⁹⁰ in foetal bone and teeth in which there was no significant difference in 43 specimens. The elevation in Sr⁹⁰ in exfoliated teeth was linear from 1951 to 1954 (0.180 to 0.581 $\mu\mu\text{c/gm}$ of Ca). A promising method for calculating the amount of tooth formed prior to birth was presented. No apparent difference was noted between Sr⁹⁰ content in teeth from breast fed and bottle fed children, but as the author points out, this is probably due to the fact that children were considered to be breast fed if that method of feeding had been used for one month. Some minor errors in interpretations are present because of the failure to consider secondary dentin as a source of Sr⁹⁰ in pre 1952 teeth. This paper illustrates that primary teeth can be useful as indicators of past and present uptake of radioactive elements in humans.

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Annual Grant DA-91

Report of December, 1961.

THE INFLUENCE OF FLUORIDE ON DENTIN

File No. - 4 - D2 - 3.

Progress Report on Project DA - 90.

"The relationship of defects in teeth associated with induced stresses to the histological pattern of tooth structure."

Kasloff, Z.

There has been a considerable amount of delay in getting this research activity under way due to physical alterations of the research laboratory during the past summer. Nevertheless, good progress has been made. During the period of structural alteration, equipment and supplies were procured and set up and a laboratory assistant given help and training in the laboratory to become proficient in selecting and preparing suitable tooth specimens for study.

Photomicrographic equipment was arranged and set up to satisfactorily give a 1 - 1 ratio image on film. About 600 extracted human teeth, maxillary and mandibular, anterior and posterior have been collected, cleaned and stored in water in a refrigerator. A random group of 10 teeth, freshly extracted, were inspected after treatment with the fluorescent penetrant dye and photographed. Comparative films indicated no change in the stress pattern as a result of subjecting the teeth to lower temperatures. This control served to rule out the possibility of influence on stress induction resulting from storage of specimens in the refrigerator. One hundred incisors and 100 bicuspid teeth have been selected from the pool to date for investigation, cleaned and assigned numbered storage vials. Each of these teeth have been mounted in an artificial stone matrix after being viewed through the photomicrographic assembly for the best position. In the case of the incisor teeth, 3 matrices were made for each tooth, representing the labial, lingual and proximal view. Matrices for the bicuspids were made in 4 positions, buccal, lingual, proximal and occlusal. We now have 700 specimen surfaces ready for study.

In October 1961, under provisions of the current grant, the grantee visited A. F. Forziati in Washington, D.C. to consult with him on the application of a method for quantitative measurement of tooth fluorescence. This consultation proved most helpful and included a demonstration of the tooth fluorometer developed by Forziati. Elements of this instrument have been adapted to this present study as a result. During the same month, alterations were completed in our laboratories and a dark viewing room is now available for conducting our fluorescent studies before and after cutting cavity preparations with various rotary cutting instruments and power sources. This phase of the study is now in progress.

APPENDIX G

Annual Grant DA-91

Report of December, 1961.

THE INFLUENCE OF FLUORIDE ON DIFFERENTIATION OF CALCIFIED STRUCTURES AND ON TERATOGENESIS.

Benjamin N. Kropp, Queen's University, Kingston, Ont.

I give you herewith my report on the work done to date on this project.

We first undertook to study the effects of fluoride, as NaF, on the chick embryo. To this end eggs of two varieties were used; (a) a Barred Rock x New Hampshire cross and (b) Leghorn. Although Leghorn eggs are the more desirable for experimental purposes, at the time the work was begun they were not available to us. Fertile eggs were incubated for 4 days since the embryo of this age is known to be more responsive to teratogenic and other substances than is the younger embryo. At this critical stage the eggs were injected with a 1% solution in distilled water of the fluoride. A total of about 36 eggs were also injected on days 2 and 3 with doses equal to those given the eggs on day 4. However, while records were kept of these the results did not deviate from those obtained for the control series and, therefore, they are not listed separately in the tables. By means of a micro-injection apparatus of a high degree of accuracy, the eggs were injected with graded doses of NaF: 1.75 mg, 1.50 mg, 1.25 mg, 1.00 mg, 0.75 mg, 0.50 mg, 0.25 mg and 0.10 mg. Controls for

each group were injected with a matching volume of distilled water. All eggs were candled daily and dead embryos (or dying ones) were examined for anomalous development before they were discarded. Infertility ran about 3%. One surviving embryo from each series was fixed in neutral buffered formalin or in Bouin's for later sectioning, staining and study.

Survival While it did not appear necessary to determine LD₅₀ for both varieties of eggs used, we did find that the Barred Rock-New Hampshire cross was less resistant to NaF than Leghorns. In the former 1.25 mg NaF invariably resulted in death of all embryos in 24 hours (Table I). Leghorns tolerate 1.50 mg and there were relatively few deaths at this dosage after 24 hours (Table III). This is close to the findings of Ridgway and Karnofsky, 1952, who found a maximum concentration of 1.75 mg in the 4 day embryo. In Leghorns concentrations below 1.50 mg administered on day 4 usually resulted in almost 100% survival at the end of 48 hours incubation.

Teratogenesis The incidence of anomalies in both varieties in fluorine-treated was very low, and was matched by the "spontaneously" occurring anomalies in the controls. There is no reason to believe that the very few anomalies noted in experimental animals were induced by the fluorine treatment. Previous experience with teratogenic substances has lead me and others to believe that the 4 day chick embryo is particularly sensitive, and on appropriate treatment congenital

defects can be predicted in central nervous system, eyes, and vascular development within 48 hours. This did not occur in these specimens. The possibility exists that fluoride-treated embryos that show no grossly observable malformations, may show either morphological or histochemical anomalies on study of sections of these specimens. The possibility must be examined that with this treatment there is a latency of response that can be determined by sampling incubated eggs at intervals up to hatching. It is planned to do this.

Experiments with rats.

Female rats were mated when in estrus, and pregnancy determined by the presence of sperm in the vagina, and the presence of a vaginal plug. On day 1 of pregnancy the animal received a single subcutaneous injection of aqueous NaF solution. The amount of NaF varied from 1 to 5 mg. Above 5 mg the effect both on mothers and embryos was extremely variable. A single injection of 8 mg (irrespective of body weight) was frequently fatal to the adult. No fatalities were encountered at 5 mg or less. Data are not sufficient at the moment to give the lethal dose for our strain of rats with any degree of precision, but I believe it may well work out to about 7 to 10 mg. However, in divided doses the toxicity appears to be substantially reduced. We have given rats 30 mg (in one case 50 mg) of NaF at the rate of 2.5 mg per day, without apparent ill-effects. Therefore, experiments are planned in which the pregnant rat will receive divided doses throughout pregnancy instead of a single dose.

Females were weighed at the beginning and end of pregnancy. On day 19 they were killed by decapitation, or a blow on the head. The uterine tract was examined in situ, the young with placentas removed and weighed, examined grossly for defects, and fixed in neutral buffered formalin. Table V summarizes the experimental findings to date on litter size, weights and incidence of anomalies. The fluoride treatment of the pregnant female, on the basis of these incomplete figures, would indicate

that neither mothers nor embryos are adversely affected. On the contrary, the litters of experimental animals are a little larger, and the newborn a little heavier than those of untreated controls. The incidence of anomalies, grossly observable, in experimental rats was 3 in 85 births; in controls 1 in 55 births.

A few embryos have been processed for microscopic study although all the remaining embryos have been fixed and are awaiting processing. Microscopic studies have only begun and no statements regarding findings can yet be made.

- - - - -

Conclusions must, of course, be tentative. With the dosage used there is no indication that gross developmental anomalies are induced in chick or rat embryos during the development periods studied. We have still to examine the embryos in detail for possible effects on calcified structures.

Table I

Barred Rock x New Hampshire cross eggs. Experimental. Eggs were incubated for 4 days then injected with the amount of NaF (in mg) shown. "Hours Incubated" refers to incubation period after NaF injection. The "anomalies number" column gives the number of survivors at the end of the incubation period that showed anomalies of any kind.

Series No.	Amount NaF	No. Eggs Injected	Hours Incub.	No. of Survivors	No. with Anomalies
A	1.75	12	24	0	0
B	1.50	12	24	0	0
Ia	1.50	10	24	0	0
Ib	1.25	10	24	0	0
Ic	1.00	20	24	11	0
Id	0.75	20	24	20	0
Ie	0.50	20	24	20	2
If	0.25	20	24	19	1
Ig	0.10	20	24	20	0

Table II

Barred Rock x New Hampshire cross eggs. Control.
 Distilled water replaced NaF solution, Column legends as in
 Table I. .

Series No.	No. Eggs Injected	Hours Incubated	No. of Survivors	No with Anomalies
A(c)	10	24	9	0
B(c)	10	24	10	0
Ia(c)	10	24	10	0
Ib(c)	10	24	9	1
Ic(c)	10	24	9	0
Id(c)	10	24	10	1
Ie(c)	10	24	8	0
If(c)	10	24	10	0
Ig(c)	10	24	9	0

Table III

Leghorn eggs. Experimental. Eggs were incubated for 4 days then injected with the amount of NaF (in mg) shown. "Hours incubated" refers to incubation period after NaF injection. The "Anomalies Number" column gives the number of survivors that showed anomalies of any kind.

Series No.	Amount NaF	No. Eggs Injected	Hours Incub.	No. of Survivors	No. with Anomalies
IIa	1.50	20	48	17	0
IIb	1.25	20	48	19	1
IIc	1.00	20	48	20	2
IId	0.75	20	48	20	0
IIe	0.50	20	48	19	0
IIf	0.25	20	48	20	0
IIg	0.10	20	48	19	1

Table IV

Leghorn eggs. Control. Distilled water replaced NaF solution.
Column legends as in Table III.

Series No.	No. Eggs Injected	Hours Incubated	No. of Survivors	No. with Anomalies
IIa(c)	10	48	10	1
IIb(c)	10	48	8	0
IIc(c)	10	48	10	0
IId(c)	10	48	9	0
IIe(c)	10	48	10	1
IIf(c)	10	48	10	0
IIg(c)	10	48	10	0

Table V.

Rats. Hooded strain. Experimental.

Pregnant females were injected subcutaneously with the amount of NaF shown in 1% aqueous solution, on 4th day of pregnancy. Females were killed at end of day 19 of pregnancy.

Female Rat No.	Dosage in Ng	Size of Litter	Average Wt of Embryos in Grams	Average Wt of Placentas in Grams	Anomalies
6	1	10	2.04	0.55	none
8	1	6	2.43	0.48	*
3	3	11	2.42	0.44	none
1	3	11	2.32	0.35	none
12	3	12	2.45	0.39	none
4	3	10	1.60	0.38	**
9	4	12	2.62	0.45	***
5	5	13	1.74	0.34	none

* = One placenta without embryo, the latter presumably resorbed in utero.

** = One embryo with umbilical hernia. One embryo with brain defect.

*** = One embryo with umbilical hernia.

Table VI.

Rats. Hooded strain. Controls.

Pregnant females were injected subcutaneously with a volume of distilled water equal to that given the experimental rats receiving the highest dosage of NaF. Females were killed at the end of day 19 of pregnancy.

Female Rat No.	Size of Litter	Average Wt. of Embryos in Grams	Average Wt. of Placentas in Grams	Anomalies
1(c)	10	2.30	0.31	none
2(c)	10	2.17	0.32	none
3(c)	9	2.21	0.29	*
4(c)	7	2.16	0.38	none
5(c)	9	2.17	0.33	none
6(c)	10	2.20	0.27	none

* = One embryo with umbilical hernia.

KROPP, B.N. and A. TRAVILL*, Laboratory of Histology and Embryology, Queen's University, Kingston, Ontario.

Dental effects in off-spring of pilocarpine-treated pregnant rats.

In a closely inbred strain of hooded rats pregnant females were injected subcutaneously with pilocarpine from the first day of pregnancy daily to term. Two series of pregnant females were used, one received a daily dose of 100 mg of pilocarpine per kg body weight, the other 150 mg. Following birth the young were weighed, then sacrificed by decapitation at one day intervals up to 10 days. The mandibles were removed, split in the mid-sagittal plane, and fixed in neutral buffered formalin. Each lateral half of the jaw was cut serially in the sagittal plane, and controls were similarly treated. The main axis of the incisors of each jaw was measured and this dimension compared in experimentals and controls of the same age. In newborn rats from treated mothers the incisor length averaged 5.55 mm, in controls 2.64 mm. The width of the teeth did not appear affected. Morphological abnormalities were not apparent in cells of epithelial layers or pulp of affected teeth. However, PAS and Mallory-stained sections indicated deficient secretion especially by the ameloblast layer in the off-spring of pilocarpine-treated mothers. Molars of young from treated mothers were normal in size. The two dosages used were equally effective in producing the results described.

TRAVILL*, A, and B.N. KROPP, Laboratory of Histology and Embryology, Queen's University, Kingston, Ontario. Weight of young rats following pilocarpine, salivary gland extract, and saliva injections.

Previous studies lead us to investigate the systemic effects on the newborn of rats treated during pregnancy with a sialagogue, and on young treated in the neonatal period with salivary gland extract and with saliva. When pregnant females of an inbred hooded strain of rats were injected daily throughout pregnancy with 100 mg pilocarpine per kg body weight, there was no significant difference in birth weight of the newborn compared with control litters. However, beginning with day 1 the weight gain in experimental neonates lagged significantly behind that of controls. This trend was maintained to day 8 when the average weight of experimental animals was 13.7 gm while that of controls was 18.5 gm. After day 8 the weight of experimentals fell precipitately and the loss in the next 24 hours was of the order of 2 gm. After this they became moribund and died on day 10. Two additional series of normal newborn rats were injected with (a) salivary gland extract adjusted so that each animal in this group received one gm of gland wet weight per kg body weight and (b) an arbitrary sub-lethal dose of 0.01 ml of human saliva. Both groups confirmed the findings of others with respect to the inhibitory effects on growth. We found the retardation to be of the order of 20%.



RAT - LITTER "A"

FIG.8

2 Litter Mates at 7 days.-

No. 7 (left) Treated with salivary gland extract daily.

Note:- eruption of lower incisors.
Stunting.

No. 10 (Right). Untreated.-

APPENDIX H

Project DA-23

FORMATION OF ENAMEL MATRIX PROTEIN AS SHOWN BY RADIOAUTOGRAPHY

AFTER INJECTION OF LABELLED AMINO ACIDS

H. Warshawsky, W. Smallwood and C.P. Leblond

Department of Anatomy, McGill University

Montreal, Canada

When radioautography of the enamel organ was done 30 minutes after injection of radioactive methionine in the mouse, two radioactive zones were observed: one in the supra-nuclear region in the functional ameloblasts and the other in the adjacent preenamel. With time, the cellular reaction decreased and eventually disappeared, while the preenamel reaction intensified (Karpishka et al, 1959). The results were interpreted to mean that a protein elaborated in the cell was promptly released to the outside, thus providing material for building preenamel. Later the matrical reaction band became broader and less intense as maturation of the enamel proceeded.

However, since at 30 minutes the reaction was seen both in the cell and in preenamel, it was possible that the synthesis of matrix proteins occurred in both locations. It was therefore decided to sacrifice animals at earlier times after injection, namely 10 minutes, and it was reasoned that if the reactions were present in both locations at that time, protein synthesis would probably occur in the two places.

It was decided to carry out this investigation with amino acids different from methionine, which was used originally. Series of rats injected respectively with proline- H^3 and leucine- H^3 were used.

MATERIALS AND METHODS

Male Sprague-Dawley rats, weighing about 100 grams were given a single intraperitoneal injection of 2.5 μ c per gram of body weight of proline- H^3 or the same dose of leucine- H^3 . The animals were killed in groups at 10 minutes, 30 minutes, 4, and 36 hours after injection. The upper and lower incisor teeth were partially dissected from the head and fixed for 48 hours in Bouin's fluid. They were then trimmed of excess tissue and decalcified in Versene.

Five micra, hematoxylin-eosin stained sections, passing sagittally through the enamel organ, were radioautographed using the coating method (Markus-Kopriwa and Leblond, 1961).

Well-sectioned, moderately exposed radioautographs of teeth were chosen for quantitative estimates. Those selected showed a relatively long and uninterrupted row of longitudinally sectioned ameloblasts, as well as a fairly intact enamel matrix, particularly at the formative, or growing end of the incisor.

Quantitative methods

Counts were made over the ameloblasts and the adjacent matrix of the enamel organ. Usually the cells examined included immature ameloblasts at the formative end, functional ameloblasts in the process of secreting preenamel, and the so-called non-functional ameloblasts, which contain pigment granules within their cytoplasm. Due to artifacts of various kinds caused by sectioning, distorted areas were omitted from the counts.

Grain counts were made under an oil immersion objective using a Whipple Micrometer ocular grid to delimit a known area of tissue. The Whipple grid measured 100 x 100 μ and was subdivided into 100 x 10 μ rectangular areas. The base line of

the grid was laid along the basal pole of the ameloblasts, thus marking off a 100 μ length of ameloblasts, with successive rectangles overlying these cells. The number of grains in each rectangle was counted until the entire height of the cell had been examined.

In a similar way, the grains over the area of enamel matrix adjacent to the apex of the ameloblast were counted. This was done by placing the base line of the grid along the edge of the matrix closest to the ameloblasts, and counting progressive 100 x 10 μ areas until the dentino-enamel junction was reached.

Because of the variation in cell height, and in the width of enamel matrix, one single average was not possible. However, it was usually possible to average the counts of several 100 μ lengths of ameloblasts and matrix, provided that both cell height and enamel matrix thickness were constant over that length of enamel organ.

RESULTS

A qualitative description will be given for the proline- H^3 series, followed by a qualitative and quantitative description of the leucine- H^3 experiments.

Qualitative Results with Proline- H^3

Ten minutes after injection (fig. 1)

The entire cytoplasm of the ameloblasts was not uniformly labelled, but showed a heavily labelled region, seen as a band of silver grains about 20 μ wide, across the cytoplasm just apical to the nucleus. Only a moderate or slight reaction was seen over the rest of the cytoplasm and the nuclei.

The matrix showed no reaction at all throughout its entire length.

Thirty minutes after injection (Fig. 2)

Extending for a short distance from the formative end of the tooth, a heavy band of radioactivity was seen between the nuclei of the ameloblasts and their apices. Beyond this point the cells showed two discrete bands of radioactivity; one in a supra-nuclear position, and a second located at the very apex of the cells and extending into the Tomes processes. The two bands of heavy radioactivity were separated by a narrow band which was only moderately labelled.

The basal region, as well as the nuclei showed only a slight reaction.

At the extreme formative end of the tooth, the very thin band of matrix was heavily labelled. As the thickness of the matrix increased, the labelled band widened and became more diffuse, but remained limited to the area of matrix in contact with the cell apices (which is here the preenamel). Towards the incisal end the band of radioactivity was quite narrow, but scattered grains were seen to extend further towards the dentino-enamel junction.

Four hours after injection (Fig. 3)

At 4 hours, a uniform though slight degree of labelling was seen over the entire cytoplasm of the ameloblasts. At some points two more intense bands of activity could still be recognized, but this was not as clear as in the 30-minute interval.

Again, the nuclei showed only slight reaction intensities.

The thin ribbon of enamel matrix at the extreme formative end was heavily labelled. As the matrix increased in thickness, the heavily labelled band also increased to a thickness of about 30 μ at the middle portion of the tooth, then decreased gradually to about 10 μ in thickness at the extreme incisal end.

On the dentinal side of the heavily labelled matrix, there was a decreasing gradient of radioactivity.

Thirty-six hours after injection (Fig. 4)

At this late time interval only a slight and diffuse reaction occurred over the cytoplasm of the ameloblasts. Towards the incisal end the reaction seemed, as in the 10-minute interval, to be confined, though much less distinctly, to a supra-nuclear band of the cytoplasm. The nuclei showed a very slight reaction intensity.

The reaction occupied a greater thickness of enamel matrix than at 4 hours. Throughout the labelled area, a slightly heavier reaction band was seen close to, but not immediately adjacent to the ameloblasts. In general, the reaction seemed less intense than at 4 hours. Since it is known that no labelled amino acids remain in the circulation after one or two hours, the increased thickness and diffuse nature of the reaction, may indicate that an intramatrix expansion causes the labelled matrix to occupy a greater area.

Qualitative and Quantitative Results with Leucine-H³

Qualitatively, the leucine-H³ series showed precisely the same patterns of silver grain distribution (Figs. 5, 6, 7). Slight differences were noted regarding the intensity of the reactions over the ameloblasts. With leucine-H³, the reactions over the cells were more intense at 4 hours (Fig. 6) and 36 hours (Fig. 7) than the reactions seen with proline-H³ (Figs. 3 and 4).

Quantitatively, the average grain counts over areas of immature ameloblasts (without matrix), at the formative end, and of active secretory ameloblasts (with matrix) for the 10- and 30-minute, 4- and 36-hour time intervals, were recorded in Table I and Figure 8. Each count represents the average of between 200 and 600 μ lengths of ameloblasts, recorded per 100 x 10 μ area. Individual counts

are given for each $10 \pm$ height of the cell, starting at the base of the cell and extending to the apex. The counts over the matrix are similarly shown, extending from the cell surface to the dentino-enamel junction. The positions of the various bands of radioactivity described above, are indicated by underlining of the numerical values.

While little change is seen in the reaction of immature ameloblasts with time, the secretory ones show at first a supra-nuclear reaction (10 minutes) and later another one at the extreme apex as well as over preenamel (30 minutes), with more accumulation in the matrix at 4 hours and diffusion of the reaction by 36 hours.

DISCUSSION

Mechanism of Enamel Matrix Formation as Shown

by the Behaviour of Secretory Ameloblasts

Thirty minutes after the injection of both leucine- H^3 and proline- H^3 , the qualitative and numerical data confirm, with more precision, the results of Karpishka et al (1959). Reactions were seen in a supra-nuclear region of the cell and at the very apex including the Tomes processes. Reactions were also seen within the preenamel matrix. To decide which of these sites, or whether all of them were synthesizing proteins, it was necessary to work at an earlier time interval, that is, at 10 minutes after injection.

The reactions observed 10 minutes after injection with both proline- and leucine- H^3 (and also confirmed with methionine- H^3), were localized over only one of the above sites, that is, the supra-nuclear region of the cell. Thus, it seems

that proteins are actively synthesized within the supra-nuclear zone of the cell. However, they would migrate very rapidly to the apical region and into the Tomes processes, and by 30 minutes some of the labelled proteins have already been secreted as enamel matrix.

The fact that a less radioactive band is located between the supra-nuclear and apical reaction zones at 30 minutes indicates that the migration from the supra-nuclear to the apical region must be rapid.

Precisely what cytoplasmic organelles are responsible for the reactions seen at 10 minutes can only be surmised from electron micrographic, biochemical and radioautographic studies. The cytoplasm between the nucleus and the cell apex is occupied by numerous elements of the endoplasmic reticulum or ergastoplasm as well as by the Golgi apparatus (Reith, 1960, 1961). From radioautographic and biochemical studies, the ergastoplasm has been implicated as the site of protein synthesis (Siekevitz and Palade, 1960). Radioautographic results with the pancreas (Warshawsky and Leblond, 1961) indicate that proteins are synthesized in the ergastoplasm and migrate to the Golgi apparatus, accumulating there at 30 minutes after injection. It is possible that a similar phenomenon exists in the ameloblasts, but since the Golgi apparatus and the ergastoplasm are in a similar location, the supra-nuclear band may be due to radioactivity in the ergastoplasm at 10 minutes and in the Golgi apparatus at 30 minutes. Thus, the passage from one structure to the other cannot be distinguished.

With time, at 4 and 36 hours, the reaction within the cells becomes more diffuse and decreases in intensity.

Nature of the Proteins Synthesized by the Ameloblasts

Since leucine is a commonly occurring amino acid in many different proteins, it is incorporated into the enamel matrix proteins as well as into the endogenous proteins and enzymes of the ameloblasts. Thus, after secretion of the enamel matrix (by 4 or 36 hours), much radioactivity is still retained in the cell. However, proline is a less widely distributed amino acid, occurring in significant proportions mainly in collagen and enamel matrix (Piez, 1961). Since the reactions over the ameloblasts at 4 and 36 hours are rather light, it is suggested that proline is used mainly for synthesis of enamel matrix.

TABLE I

RADIOACTIVITY (EXPRESSED AS GRAIN COUNTS) IN REPRESENTATIVE AREAS
OF AMELOBLASTS AND MATRIX AT VARIOUS TIMES AFTER LEUCINE-H³ INJECTION

Time after injection	Immature ameloblasts (no matrix)				Secretory ameloblasts (and matrix)									
	Base		Apex		Base		Apex		Matrix					
10 minutes	14	19	46	20	13	16	42	47	23	11	-			
30 minutes	21	27	34	30	15	10	19	26	16	26	31	50		
4 hours	37	42	53	43	30	25	33	31	32	32	29	86	55	38 24
36 hours	16	19		29	14	15		17	17	19	20	28	31	30 30 18

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LEGEND OF FIGURES

A = Ameloblasts; DE = Dentino-enamel junction; PE = Preenamel; S = Stratum intermedium.

Fig. 1. Radioautograph of the incisor of a rat killed 10 minutes after injection of proline- H^3 (H & E stained, x 515).

A band of radioactivity (arrow) is present in the supranuclear portion of the ameloblasts.

The nuclei, preenamel and enamel matrix are unlabelled.

Fig. 2. Radioautograph of the incisor of a rat killed 30 minutes after injection of proline- H^3 (H & E stained, x 515).

Two bands of radioactivity (arrows) are present at this time. One occurs in the supranuclear position of the ameloblasts; the second is found at the cell apex, and over the preenamel.

Fig. 3. Radioautograph of the incisor of a rat killed 4 hours after injection of proline- H^3 (H & E stained, x 515).

A slight reaction persists over the ameloblasts, but the enamel matrix is heavily labelled (arrow).

Fig. 4. Radioautograph of the incisor of a rat killed 36 hours after injection of proline- H^3 (H & E stained, x 515).

A light reaction is still present over the ameloblasts. The reaction over the enamel matrix is wider, but somewhat more diffuse (arrow).

Fig. 5. Radioautograph of the incisor of a rat killed 30 minutes after injection of leucine- H^3 (H & E stained, x 380).

Two bands of radioactivity can be seen (arrows); one located

supranuclear, the other at the cell apex and in the preenamel.

Fig. 6. Radioautograph of the incisor of a rat killed 4 hours after the injection of leucine- H^3 (H & E stained, x 380).

The ameloblasts are more uniformly, though still heavily labelled. The heaviest reaction is seen over the enamel matrix (arrow).

Fig. 7. Radioautograph of the incisor of a rat killed 36 hours after injection of leucine- H^3 (H & E stained, x 380).

The ameloblasts are still quite strongly labelled. The enamel matrix is uniformly, though heavily labelled (arrow).

Fig. 8. Schematic representation of the reaction in immature (left) and secretory ameloblasts (right) at various times after leucine- H^3 injection.

There is little change with time in the immature ameloblasts, whereas the supranuclear reaction seen at 10 minutes in secretory ameloblasts is followed at 30 minutes by a reaction in the matrix close to the cells. The reaction is later found farther in the matrix.

FIG. 1.

10 MIN.



FIG. 2.

30 MIN.



FIG. 3.

4 HRS.

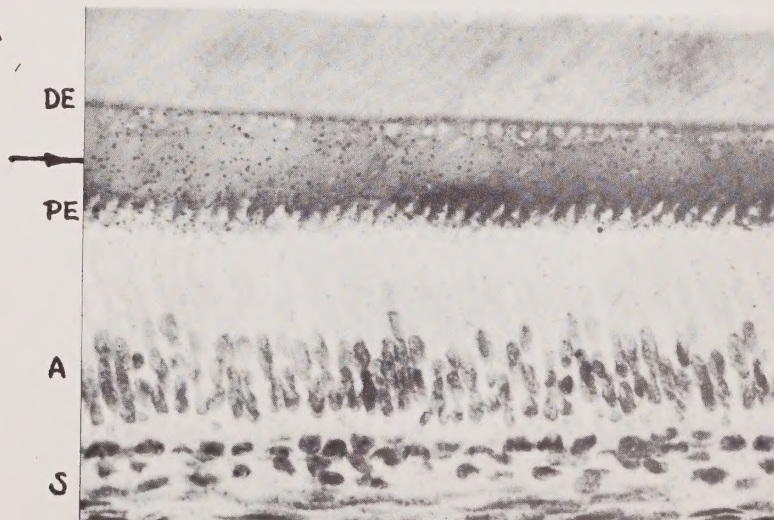


FIG. 4.

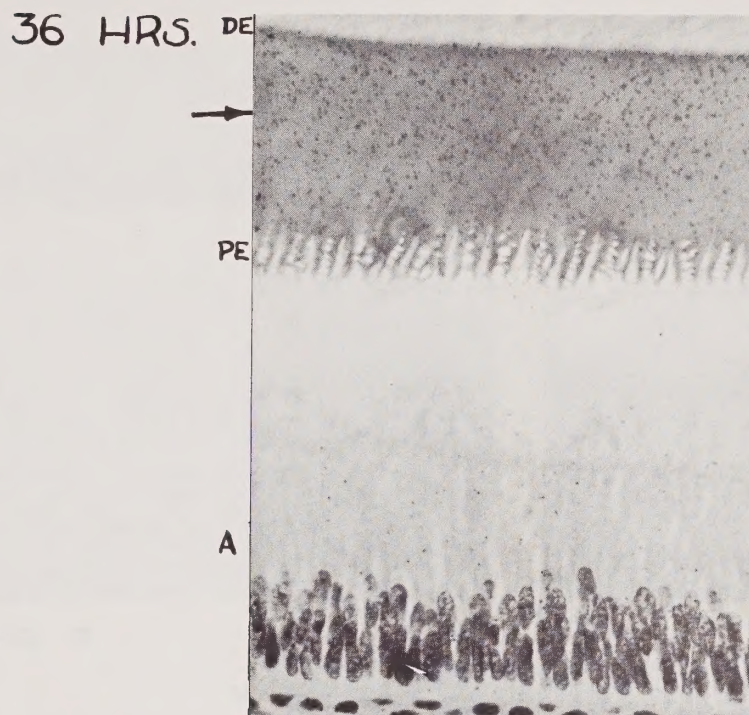


FIG. 5.

30 MIN

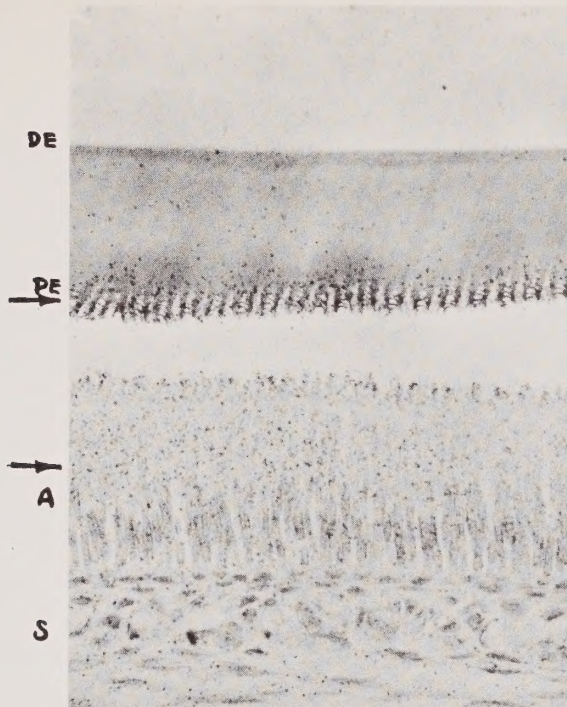


FIG. 6.

4 HRS.

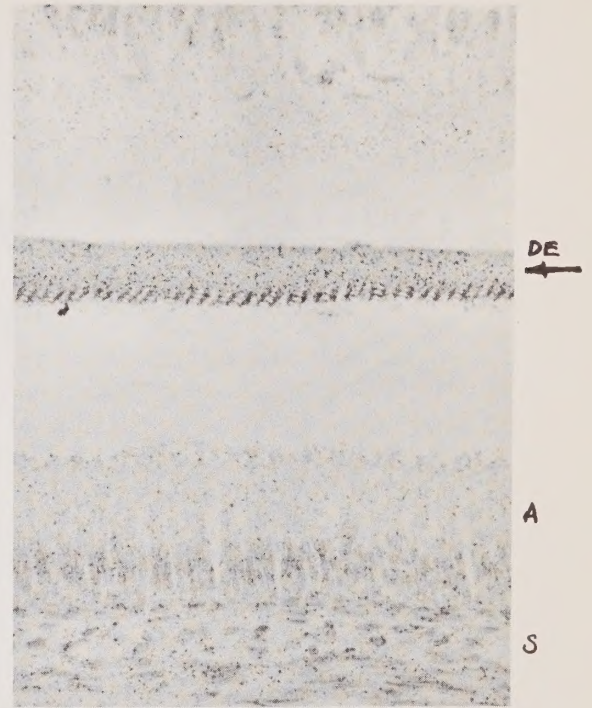


FIG. 7.

36 HRS.

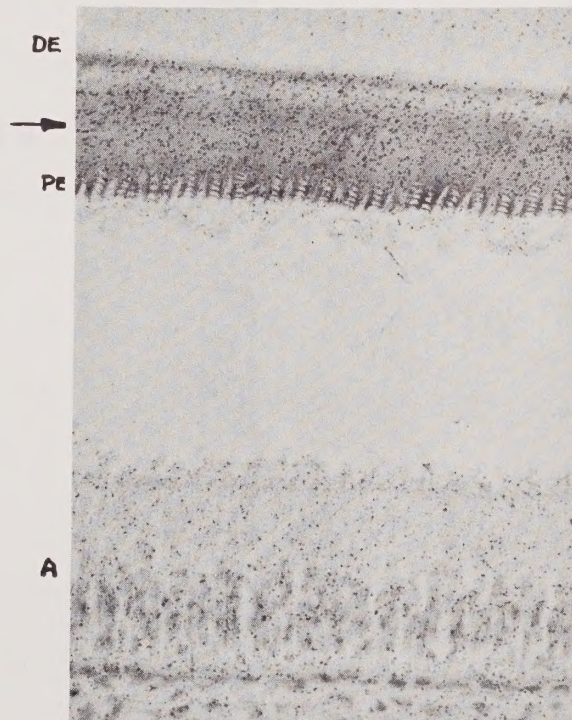
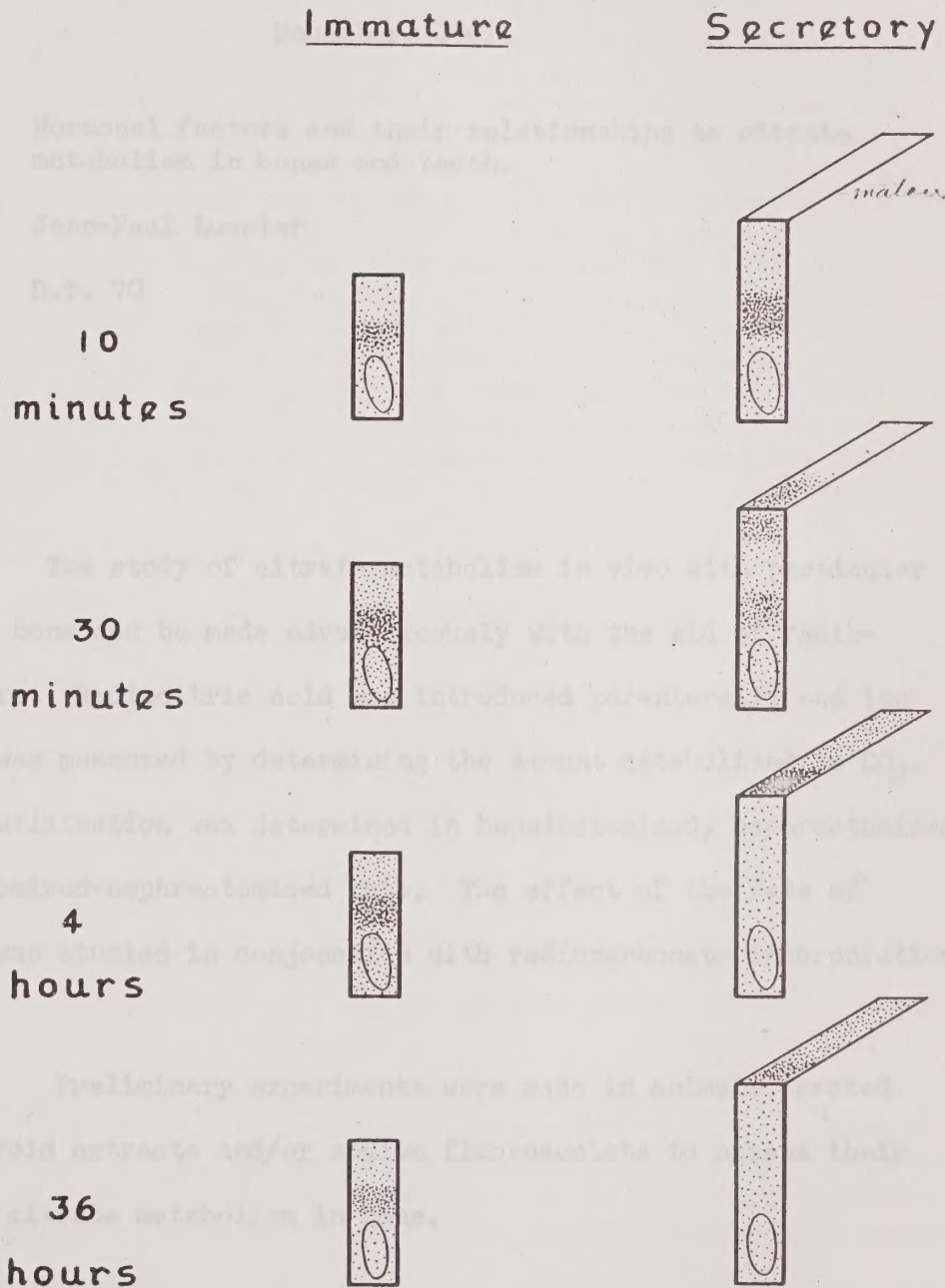


Figure 3



APPENDIX I

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

NATIONAL RESEARCH COUNCIL

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Subject: Hormonal factors and their relationships to citrate metabolism in bones and teeth.

Grantee: Jean-Paul Lussier

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SUMMARY

The study of citrate metabolism in vivo with particular reference to bone can be made advantageously with the aid of radioactive tracers. Radiocitric acid was introduced parenterally and its utilization was measured by determining the amount catabolized to CO_2 . The rate of utilization was determined in hepatectomized, nephrectomized and hepatectomized-nephrectomized rats. The effect of the rate of utilization was studied in conjunction with radiocarbonate incorporation in bone.

Preliminary experiments were made in animals treated with parathyroid extracts and/or sodium fluoroacetate to assess their influence on citrate metabolism in bone.

INTRODUCTION

Most of the knowledge we have acquired on bone dynamics has been achieved through studies of calcium and phosphorus metabolism. The organic compounds of bone remained in the shade until Dickens (1) in 1941 discovered that citrate was present in rather large quantities in bone. This contribution was followed by many others which established the occurrence of citrate in the different organs and fluids of the organism (2)(3) of many animal species (4). More recent attempts have brought information on the presence of other organic acids in bone (5).

As bone is known to adsorb and retain many compounds or elements circulated by the blood in normal or pathological conditions, the question was raised as to where the high content of citrate in bone is maintained. It was shown by experiments in vitro that bone crystals exchange citrate preferentially for trivalent anions (6). Therefore, as citrate is a normal constituent of blood and as bone crystals can adsorb citrate on the surface through an exchange process, the accumulation of citrate may be the result of non vital mechanisms.

However, as shown by Dickens (1), and later by Newman et al, (7) parathyroid extracts stimulate citrate production in bone. These observations together with some others made in relation to the effect in vitamin D on bone citrate (8), rendered possible the hypothesis that bone cells were capable of producing large quantities of citrate in foci of bone resorption.

Dixon and Perkins (9)(10) have identified enzymes in bone responsible for the production of citric acid namely citrogenase and aconitase. Recently Laskin and Engel (11) have followed in vitro citrate synthesis by bone slices confirming that bone pretreated with PT extracts was able to produce more citrate from pyruvate and showed other signs of citrate accumulation (as depletion of glycogen stores, decreased activity of succinic dehydrogenase and an overall decreased rate of aerobic carbohydrate degradation). The interest of these findings is linked to their ability to elucidate how citrate intervenes in bone dissolution through chelation which is an essential step in bone resorption. This point reemphasizes the relationship between citrate production and calcium metabolism.

From this aspect some contributions have been brought which indicate the interdependency of calcium and citrate concentration in the blood and in the urine (12). But the picture is complicated by the fact that the concentration of citrate in the blood is dependent not only on the absorption and excretion of preformed citrate and its deposition in bone, but also on the relative rates of its formation and degradation within the tissues.

In the state of knowledge of this question we have thought we could bring a contribution in working along the following hypothesis.

PURPOSE OF THE PROJECT

It was intended in the present project to study in vivo the exchange reactions of citric acid labelled with radiocarbon in bone

tissue under specific experimental conditions. Thus by introducing parenterally a tracer amount of radiocitric acid it was expected that one part would reach directly the bone tissue and become part of it a) through adsorption in the crystals and b) through exchange with other surface ions and that another part after following the metabolic pathways would reach bone as some intermediary or end-products and penetrate it through the same exchange process. In view of the complications to identify the intermediary products in bone, we have not included them in the scope of the actual experiments. The end-product of these metabolic reactions, carbon dioxide, became one of the main objects of our interest.

The follow-up of citric and carbonic acids was undertaken under the following conditions:

- a) when bone tissue was overproducing citrate,
- b) when the whole organism was saturated with citrate either by lack of utilization or by overproduction.

In order to reproduce these conditions, animals were treated accordingly with parathyroid extracts and/or with sodium fluoroacetate. In another series of experiments the same observations were attempted in nephrectomized and/or hepatectomized animals.

EXPERIMENTALS

In a first series of experiments male white rats of the Wistar strain weighing about 70 grams (60-80) were treated in the following way:

a) Five rats were injected subcutaneously with parathyroid extracts (x) 50 units at least 4 hours previous to the intraperitoneal introduction of citric acid.

b) Six rats were injected with 0.5 ml and two with 0.25 ml of a 10m M solution of sodium fluoroacetate per hundred grams of body weight four hours previous to the intraperitoneal introduction of citric acid.

c) Six rats were injected with 50 units of the parathyroid extracts and 0.5 ml of a 10m M solution of Na fluoroacetate per hundred grams of body weight four to six hours previous to the intraperitoneal introduction of citric acid.

d) Three rats were used as controls and received none of these treatments. All animals were injected with one ml of a solution containing one milligram of radioactive citric acid labelled with carbon 14. The specific activity of this compound was 7.0 microcuries per milligram. According to the supplier's (+) specifications the radioactivity was carried in the carboxylic groups 1 or/and 5. The amount of citric acid introduced was kept low on purpose. Assuming that the plasma citrate is about 5 to 6 milligrams per cent and assuming that the extra cellular fluid has the same concentration, the injection of 0.5 to one milligram of citric acid should not increase the actual citricemia by more than thirty to sixty per cent (citricimia amounts to eight to ten milligrams per cent). These variations can be considered in the range of rapid and normal adjustment abilities of the animals without introducing important side effects.

(x) Eli Lilly's Parathyroids, U.S.P. 100 units per cc.

(+) New England Nuclear Corporation. Citric acid C. 14, 1-5, 142 mg/mc

Following the injection, each animal was placed in a metabolic cage for the duration of the experiment. The expired CO₂ was collected in 20 per cent NaOH. The resulting carbonate was precipitated with barium chloride, centrifuged, washed, dried, weighed, plated and counted under a thin window GM counter. (x)

SURGICAL PROCEDURES

The removal of organs was done under ether anesthesia. Hepatectomy was partial as the posterior lobe was left in place. Through this procedure however eighty per cent of the liver was removed. Bilateral nephrectomy was performed through abdominal approach. Nephrectomy on the hepatectomized animal was done two days after hepatectomy. There has been no mortality due to surgical procedures. The nephrectomized animals showed no sign of discomfort. The hepatectomized-nephrectomized animals had a lower skin temperature, but otherwise were in good conditions.

BONE SPECIMENS

From the hind limbs, tibias and femurs were removed, wrapped in parawax and placed in a freezer at -20° C.

At the time of processing the bones for chemical determinations, they were cleaned of skin, flesh and tendons. The epiphyses were broken at the level of the cartilage plate and discarded. Bones were then dried in vacuo at 70° C, weighed and processed for CO₂ determinations in a Van Slyke apparatus with a combustion tube and a three line manometric chamber.

(x) At the beginning of the experience the radiocitric acid used had a specific activity of 0.7 mc/mM; aliquots of 2 cc of the solution containing

CO₂ DETERMINATIONS

Bone specimens were introduced in the combustion tube which was connected to the Van Slyke apparatus. Five ml of H₂SO₄ 20% were introduced after the gases had been expelled from the apparatus. One ml of NaOH 0.5N was used to entrap the evolving CO₂. The tube was heated; boiling was maintained for six minutes which appeared to be a safe period for complete removal of CO₂ from the solution. The measurements were then completed according to the standard technic.

The resulting mixture of the manometric chamber was recuperated. An aliquot of one ml was placed in a planchet, evaporated and counted. Calculations were made as follows:

$$(P_1 - P_2 - b) \times F \times 3.66 = N \text{ mg CO}_2$$

$$P_1 - P_2 = P \text{ CO}_2$$

$$b = P \text{ CO}_2 \text{ in blank}$$

$$F = \text{Van Slyke Factor for Carbon Calculation}$$

$$3.66 = \text{conversion constant for carbon to carbon dioxide value.}$$

CITRIC ACID DETERMINATIONS

Citric acid determinations were made according to Carlson et al (8) on samples of the bone solution. The determination of radioactive one milligram were used. The next batch of radiocitric acid had a specific activity twice as high as the first one. From then on, the animals were injected with one cc of solution containing 0.5 milligram, Thus the amount of the radioactive isotope injected was kept in the same range.

citric acid and other organic acids in bones of small dimension as tibias or femurs of rats weighing less than a hundred grams, has presented a problem which has not yet been solved.

One alternative is to remove sulfuric acid used for dissolving bone in the Van Slyke apparatus by means of ion exchange on plastic resins. The large ratio of sulfuric acid to citric acid in the solution theoretically renders impossible the removal of the first acid without the removal of the second. No attempt was made to develop this method.

Another alternative was to use the alternate bone (the right tibia for instance when the left was used in the Van Slyke apparatus) in the following way: to decalcify by means of EDTA, plate and count. Again it was found that the amounts of radioactive materials were too low to be accurately determined by the GM counter.

A third alternative was to use a combustion chamber in connection with an ion chamber. In this set up, the sulfuric acid can be oxidized; the CO_2 evolved can be trapped in an ion chamber and the radioactivity of the gas is determined on an electrometer. This procedure was adopted. It has been found that the amount of radioactivity present in the bone samples was within the limits of accuracy of the Nuclear Dynacon. Preliminary tests have been found satisfactory. The right oxidative mixture has still to be selected since the one proposed by the authors of the method is not applicable to our particular conditions.

The determination of radiocitric acid in bone cannot be made specifically with the technic above described. It is expected that the radioactivity of the organic compounds in bone tagged with radiocarbon will appear in the resulting CO_2 . To assess the right distribution of radiocarbon among the organic compounds of bone, it is proposed to inject some animals in each group with higher amount of radiocitric acid and to make a chromatographic survey of organic acids according to the technic previously described (14) and with the aid of a Nuclear automatic scanner. The present report will not mention the technical procedures related to the use of these apparatus which are close adaptations of the manufacturer's instructions. We shall mention the decontamination apparatus made and used in our laboratory for the elimination of radioactive CO_2 from the ion chamber after each determination. (Figure I)

It seems also opportunate to indicate the use of the apparatus shown in Figure 2, which is intended to improve the determination of the specific activity of respiratory gases. The animal is placed as usual in a large dessicator.(A). By means of polythene tubing and a circulating pump (B) the gases are driven out of the dessicator into a liquid air trap (C). At this stage CO_2 freezes; the other gases continue the trip to the other parts of the apparatus. After a determined period, the Dewar flask (D) is removed; the CO_2 melts and is attracted by a mild vacuum to the ion chamber (E) where the recording of the radioactivity is done simultaneously. The CO_2 is then trapped in a solution of NaOH (F). Samples from this solution are used for the determination of carbonate produced during the concerned interval.

RESULTS

I- Effect of parathyroid extract and/or sodium fluoroacetate on the production of respiratory carbon dioxide.

1- Respiratory conditions.

Male rats weighing 70-80 grams normally produce carbon dioxide at the rate of 1.10 gram per hour when expressed in grams of barium carbonate. When treated with fluoroacetate or parathyroid extracts they show a decreased respiratory function. We have not in this experiment established if the cause of the lower production is located at the cellular level or is related to agents of transportation from the cells to the lungs. Consequently, the amount of the radioactive CO_2 present in the respiratory gases is proportionally diminished. To assess the data from the treated animals on the basis of the normal controls, the values were corrected by way of a multiplying factor determined in the following manner:

$$\left(\frac{N - T}{T} \right) \times 100 + 1.00 = F, \quad \text{where } N \text{ is the value of the normal controls,}$$

T the value of the treated group and F the resulting multiplying factor.

2- Respiratory data.

After the corrections were made, it became obvious that the parathyroid extracts have not operated as an effective agent in stimulating or inhibiting the utilization of citrate at the peripheral tissues, as shown in Table I. When fluoroacetate was injected in doses of 2.5 mg/kg of body weight no influence was noticeable on the rate of

citrate utilization. However an injection of 5.0 mg/kg rendered the action of fluoroacetate very obvious and the amount of radioactive CO_2 in the respiratory gases fell to about $1/3$ of the control value, during the first hour following the injection of radiocitric acid. The combination of parathyroid extracts and fluoroacetate further decreased the rate of utilization of citrate but not significantly more than with fluoroacetate alone.

II- Effect of parathyroid extract and/or sodium fluoroacetate on bone carbonate.

The content of carbonate in the tibias of animals treated with fluoroacetate or/and parathyroid extracts did not vary, according to the data presented in Table II. This observation assessed that our experimental conditions from the standpoint of ionic concentrations were such as to cause no disturbance in the ionic equilibrium of the extracellular fluid and consequently in the crystal content in carbonate.

The measurements of the radioactivity of the CO_2 issued from bone presented the disadvantage of being too close to the background and therefore of low statistical value. Interpretation must then be very cautious. However, if we care to analyse the possible meaning of these data, we may find that the treated animals, whatever group they belong to, generally came with lesser values than the controls. This again may be due to the differences in the respiratory or circulatory rate between the control and the treated animals. Once the corrections were made with the same F coefficients as listed in Table I, the values were much closer to the normal ones and appeared to be statistically non significant.

III- Effect of parathyroid extract and/or sodium fluoroacetate on bone citrate.

Table III shows that the animal treated with fluoroacetate did not appreciably differ from the normals. The introduction of five milligrams of fluoroacetate/kg four hours before the injection of citric acid had no effect whether the animals were previously treated with the parathyroid extracts or not. It is somewhat disturbing to find that the animals treated with parathyroid extracts have a lower concentration of citric acid in bone when compared to the controls. Although it is not statistically significant, the trend is quite obvious. It remains that the inhibitory action of the fluorotricarboxylic acid concealed all general and local influences that could be produced by the parathyroid extracts. Under this respect, we must rewrite the statement of Beaulieu and Dallemagne (13) that: "l'acide citrique tire peut-être son origine des processus glycolytiques, mais on pourrait également émettre l'hypothèse qu'en ce qui concerne l'intervention du fluoroacétate, le squelette se comporte comme le foie au niveau duquel le produit toxique ne se transforme pas en fluoroacétate dérivé intervenant pour provoquer l'accumulation de l'acide citrique".

IV- Effect of hepatectomy, and/or nephrectomy on the production of respiratory carbon dioxide.

The representation of cumulative CO₂ in the different experimental groups is shown in figure 3. The columns representing the CO₂ production in the hepatectomized animals show that a plateau is reached at the third hour. At that time 55 to 60% of the radioactivity injected has been recovered in the respiratory CO₂. This is well in accordance

with the values already found for the normals (15). Therefore, no mention of the normal values has been made in these histograms as the normal controls and the hepatectomized animals are considered as identical.

The columns representing the CO₂ production in the nephrectomized animals show a continuous increase of CO₂ production during the six hours following the injection of radioactive citric acid. The absence of the kidneys delays the citrate utilization, this is what is conspicuous in the three first hours. However the utilization is brought to a further extent than in the hepatectomized animals as no citric acid is getting access to the urine. The proper effect of nephrectomy in terms of water disbalance, accumulation of metabolic end-products and other disturbances may delay the incorporation of tagged intermediary products into fat or protein synthesis and thus permit a more extensive degradation of citric acid to carbon dioxide. This is further substantiated by the fact that in figure 4, at the sixth hour, the nephrectomized are producing CO₂ in significantly greater quantities than the hepatectomized at the 5% level. The points of the two first hours have also the same degree of significance.

In the case of the hepatectomized-nephrectomized animals, the rate of CO₂ production is very low. The explanation just given for the lower rate of CO₂ production in the nephrectomized is also valid for the hepatectomized-nephrectomized ones; although in the latter, the saturation point seems to be reached as the curve in figure 4 shows that the utilization of citric acid is maintained maximal for the first

four or five hours.

The hepatectomized and the nephrectomized animals got rid of the acid injected at normal and close to normal rate respectively as shown in figure 4, oxidizing half of it in the first hour. The hepatectomized-nephrectomized animals could do better than ^{no} to utilize more than a third of the value of the other groups. A rough estimation would establish citric acid utilization in the hepatectomized-nephrectomized animals at less than one milligram per kilogram of body weight.

V- Effect of hepatectomy and/or of nephrectomy on bone carbonate.

The results expressed in Table V show that the removal of kidneys has a definite influence on bone carbonate. Whereas in the hepatectomized animals the value is close to 2.0 milligrams per hundred mgs. of dry bone, this value drops between 1.49 and 1.82 in the nephrectomized. The effect of nephrectomy is more acute in the intact animals than in the previously hepatectomized one. It seems that the agents responsible for the decrease in bone carbonate cannot be formed so freely in absence of liver.

The specific activity of bone carbonate is shown as histograms in figure 5. The radioactivity expressed in terms of counts per minute per milligram of carbon dioxide is attracted in bone and released from it at a rate which is quite similar in both hepatectomized and nephrectomized animals. However, as there is less carbonate in nephrectomized animals, this apparent similitude disappears when appropriate corrections are made. This point will be more emphasized

when values of Table VI will be discussed.

The hepatectomized-nephrectomized animals keep in the bone a level of radioactive carbonate which takes a long time to fade out as the concentration in the extracellular fluid seems to be fairly constant on account of the low rate of citrate utilization in the whole organism and the rather short periods of observation. When the specific activity is expressed in terms of counts per minute per hundred milligrams of dry bone weight, a representation close to the facts can be obtained. In table VI, values of the specific activity of the bone carbonate come to a slow rate of desorption at the third hour. This part of the process is attained in the nephrectomized rat about an hour later. In the hepatectomized-nephrectomized animal the radioactivity is kept constant for the reasons abovementioned.

In order to compare the three groups on the same basis of respiratory and circulatory conditions, the mean values were adjusted with the aid of the coefficients 1.33, and 1.40 respectively for nephrectomized and hepatectomized animals. The results are now less dramatic, but they still show that the nephrectomized animals had a rate of exchange 20% less effective than the hepatectomized; under the same conditions the hepatectomized-nephrectomized were about 50% less effective.

VI- Effect of hepatectomy and/or nephrectomy on bone citrate.

The methods for determining the specific activity of bone citrate are not yet up to the point. Until such step is successfully reached, the influence of citrate accumulated in the circulating fluids on bone cannot be evaluated.

CONCLUSIONS

1- The introduction of radioactive citric acid in animals previously treated with parathyroid extracts demonstrated that the utilization of this compound was not influenced at the level of the peripheral tissues when measuring the amount of carbon ¹⁴ in respiratory gases. Values had to be corrected for changes occurring in the respiratory and circulatory functions.

The same observation could be done in the case of animals treated with fluoroacetate in doses of 2.5 mg/kg. However, at the rate of 5 mg/kg the utilization of citrate was decreased by two-thirds.

In the animals treated with both parathyroid extracts and fluoroacetate the results were not different from those treated with fluoroacetate alone.

2- By determining the amount of carbonate in bone it could be shown that the treatments already mentioned caused no change in the carbonate content of the tibias. The specific activity of radiocarbonate in these bones were not significantly different between the groups of treated animals and the controls.

3- Bone citrate remained within the normal ranges following the PTH or/and F-acetate treatments.

4- The introduction of radiocitric acid in animals treated with parathyroid extracts could not demonstrate that PTE had increased the production of citric acid in bones. Better evaluation of this procedure will be done when the specific activity of citric acid is determined.

Carbon dioxide which is a dependable witness of the rate of utilization of citrate lead to no modification substantiating positive statements.

Individual variations within small groups of animals were sometimes too wide to ascertain a comfortable range of confidence. A more elaborate study will then be required to establish these facts with accuracy.

5- The study of the respiratory CO_2 production in reference to citrate metabolism in the hepatectomized and nephrectomized animals has established that the former are not different from the normals while the latter constitute functionally a different group. However, under the conditions used in our experiments (v.g. maximum increase of citricemia kept below 100%) the difference is only apparent as due to some circulatory or respiratory dysfunction. On the contrary when nephrectomy was performed in hepatectomized animals a considerable drop in the CO_2 production was observed.

6- The concentration of bone carbonate was found to be significantly less in the nephrectomized animals when compared to the hepatectomized. The hepatectomized-nephrectomized also had a lesser value than the hepatectomized but not as low as the nephrectomized. Nephrectomy is thought to enduce more extensive desequilibrium in the extracellular liquid of the intact animal than in the hepatectomized.

7- The retention of radioactive CO_2 in bone was more prolonged in the nephrectomized than in the hepatectomized animals. In the hepatectomized-nephrectomized animals, the retention did not reach the same level but was maintained constant for at least four hours, thus indicating that

the turnover of radioactive bicarbonate in LEC and consequently in bone was considerably reduced.

8- In comparative studies of this nature important parameters are necessarily overlooked. Generally one is inclined to study one or two variables and try to keep other factors constant. Since the normal and hepatectomized animals show a maximum incorporation of radioactive material early in the first hour after its introduction in the body, it was thought that this particular time could be selected as a constant. It was found that in the nephrectomized this does not hold, as the maximum occurs during the second hour. In the hepatectomized-nephrectomized animals, the maximum is stretched across a four hour plateau. The maximum could have been more accurately determined only if the dose injected would have been cut by half or less.

It should be kept in mind that the utilization coefficient of citrate in the nephrectomized or in the hepatectomized animals is quite the same when the dose injected is within physiological limits; doses of citrate over 10 mg/kg cannot be metabolized in the nephrectomized rat as fast as in the normal.

The hepatectomized-nephrectomized animal is a useful preparation to evaluate the utilization of citrate and perhaps other organic salts. It has been interesting to note that hepatectomy or nephrectomy per se may not induce drastic changes in the citrate utilization whereas the combination of these treatments results in a considerable reduction in the rate of utilization when the muscles, heart and viscera remain the main users of citrate. Partial hepatectomy by itself did not bring long

lasting changes in enzymatic conditions, as a matter of fact three days after the operation, the animals had recuperated enough to behave normally. Nephrectomy alone produced effects which were altered through the influence of the detoxifying actions of the liver. Concomitant hepatectomy and nephrectomy brought effects far deeper than those of the individual interventions.

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TABLE I

Amount of carbonic acid and specific activity of respiratory gases
one hour after the parenteral injection of radioactive citric acid.

Treatment	No. of animals	CO ₂ content as gms of BaCO ₃ Mean $\pm$ S.D.	Specific activity as % of injected dose Mean $\pm$ S.D.	F (x)	S.A. x F
Normal	3	1.10 $\pm$.36	A 32.0 $\pm$ 11.7	1.00	32.0
Parathyroid Extracts	4	0.90 $\pm$.14	B 26.0 $\pm$ 9.8	1.22	31.7
Fluoroacetate 2.5 mg/kg	2	0.84 $\pm$.10	C 24.3 $\pm$ 3.9	1.31	31.8
5.0 mg/kg	4	0.66 $\pm$.17	D ① 6.0 $\pm$ 1.2	1.73	10.4
Fluoroacetate 5.0 mg/kg + PTE	6	0.50 $\pm$.10	E 5.2 $\pm$ 4.3	2.50	9.0

$$(x) \quad F = \frac{(N - T)}{T} \times 100 + 1.00$$

N = value of the control group
T = value of the treated group

① no significant difference between D and E

TABLE II

Amount and specific activity of carbonate in tibias of animals treated with fluoroacetate and/or parathyroid extracts.

Group	No. of animals	Amount of carbonate in mg of CO ₂ per 100 mg dry bone Mean $\pm$ S.D.	Specific activity		Specific activity $\times$ F	
			cpm/min/mg CO ₂ Mean $\pm$ S.D.	cpm/min/100 mg dry bone Mean $\pm$ S.D.	cpm/min/mg CO ₂	cpm/min/mg dry bone
Normal	3	1.73 $\pm$ 0.48	50 $\pm$ 9.0	81 $\pm$ 9.0	50	81
PTE	4	1.62 $\pm$ 0.12	23 $\pm$ 17.0	36 $\pm$ 24.0	39.8	62.3
F-acetate	6	1.60 $\pm$ 0.21	35 $\pm$ 18.0	57 $\pm$ 31.7	42.7	69.5
PTE + F-acetate	6	1.63 $\pm$ 0.18	15 $\pm$ 27.6	24 $\pm$ 40.9	37.5	60.
		$\bar{x}$ N = 5				

TABLE III

Citric acid content of the tibia of rat following treatment in the parathyroid extracts acid/or sodium fluoroacetate.

Group	No. of animals.	Amount of citric acid mg/100 mg dry bone Mean $\pm$ S.D.
Normal	3	0.55 $\pm$.114
PTE	4	0.43 $\pm$.084
F-acetate	6	0.59 $\pm$.041
PTE + F-acetate	6	0.59 $\pm$.091

TABLE IV

Sodium carbonate collected per hour following the injection of radiocitric acid

Hours (after injection of citric acid)		I Hepatectomized		II Nephrectomized		III Hep.-Neph.		P
	N	M $\pm$ S.E.		N	M $\pm$ S.E.		M $\pm$ S.E.	
1	8	1.114 $\pm$.036		10	1.003 $\pm$.163	11	0.902 $\pm$.050	< .05
2	10	1.107 $\pm$.181		12	0.815 $\pm$.092	9	0.804 $\pm$.037	< .01
3	6	1.012 $\pm$.069		9	0.859 $\pm$.048	11	0.705 $\pm$.018	< .01
4	9	1.058 $\pm$.040		11	0.863 $\pm$.031	10	0.807 $\pm$.017	< .01
6	6	1.020 $\pm$.095		6	0.803 $\pm$.070	6	0.750 $\pm$.050	< .01

Value of normal rate: 1.107 gm/hr.

F ratio highly significant (< 1%) between Gr. I, II and III.

TABLE V

Amount of carbonate present in tibias
from hepatectomized, and/or nephrectomized animals,
in milligrams per 100 mgs of dry bone.

Hours after injection of radio citric acid	1 Mean $\pm$ S.D.	2 Mean $\pm$ S.D.	3 Mean $\pm$ S.D.	4 Mean $\pm$ S.D.	6 Mean $\pm$ S.D.
Hepatectomized	2.06 $\pm$.33	2.03 $\pm$.28	-	1.90 $\pm$.22	2.18 $\pm$.2
Nephrectomized	1.49 $\pm$.30	1.60 $\pm$.28	1.82 $\pm$.36	1.51 $\pm$.26	1.72 $\pm$.2
Hep-Nephrectomized	1.77 $\pm$.41	1.78 $\pm$.24	1.74 $\pm$.28	1.71 $\pm$.30	1.92 $\pm$.3

TABLE VI

Specific activity of Bone CO₂
per hundred grams of dry bone tissue

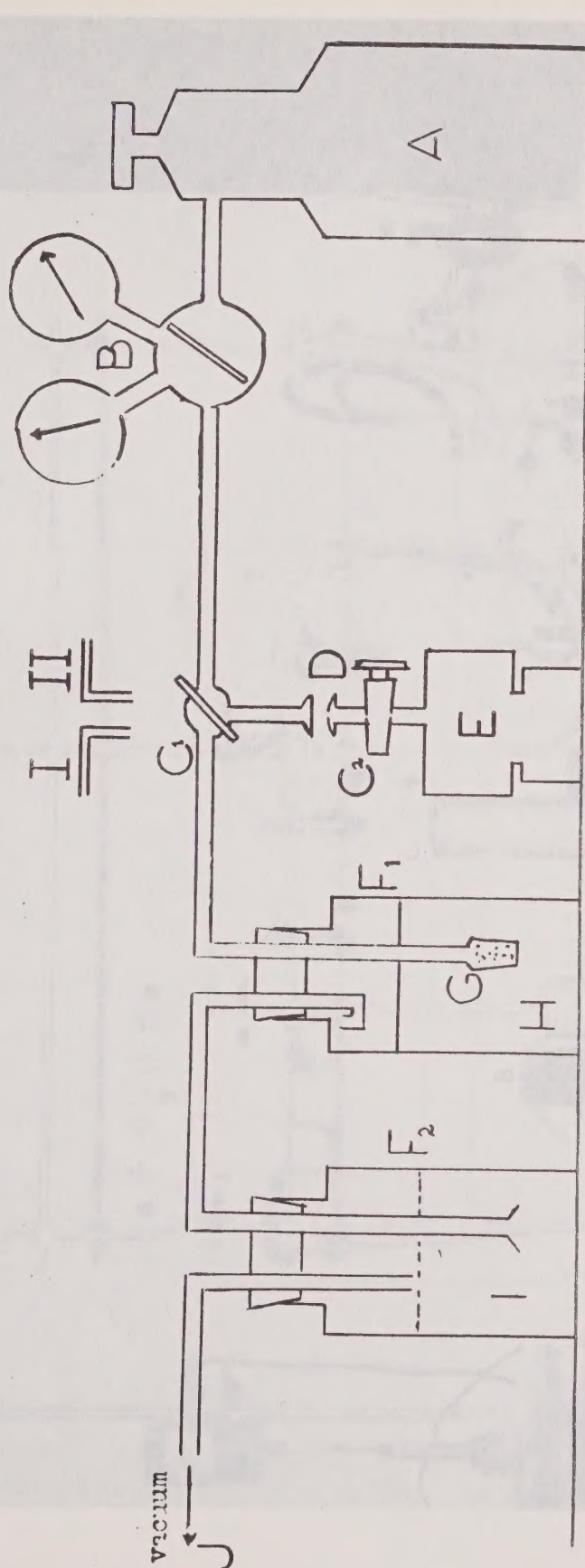
Hours after injection of radio active acid	1	2	3	4	6
H	83.7 $\pm$ 18.6	66.0 $\pm$ 27.4	27.0 $\pm$ 10.7	30.1 $\pm$ 9.8	26 $\pm$ 13
N	51.2 $\pm$ 29.1	51.2 $\pm$ 14.4	36.4 $\pm$ 15.6	22.4 $\pm$ 12.7	23.9 $\pm$ 10
HN	38.2 $\pm$ 14.1	41.8 $\pm$ 15.8	41.6 $\pm$ 18.2	40.5 $\pm$ 18.9	41.8 $\pm$ 17

Values of means after correction for
abnormal respiratory conditions

H	83.7	66.0	27.0	30.1	26
N	68.2	68	48	29	32
HN	54	58	58	59	58

FIGURE 1

DECONTAMINATING APPARATUS FOR ION CHAMBER

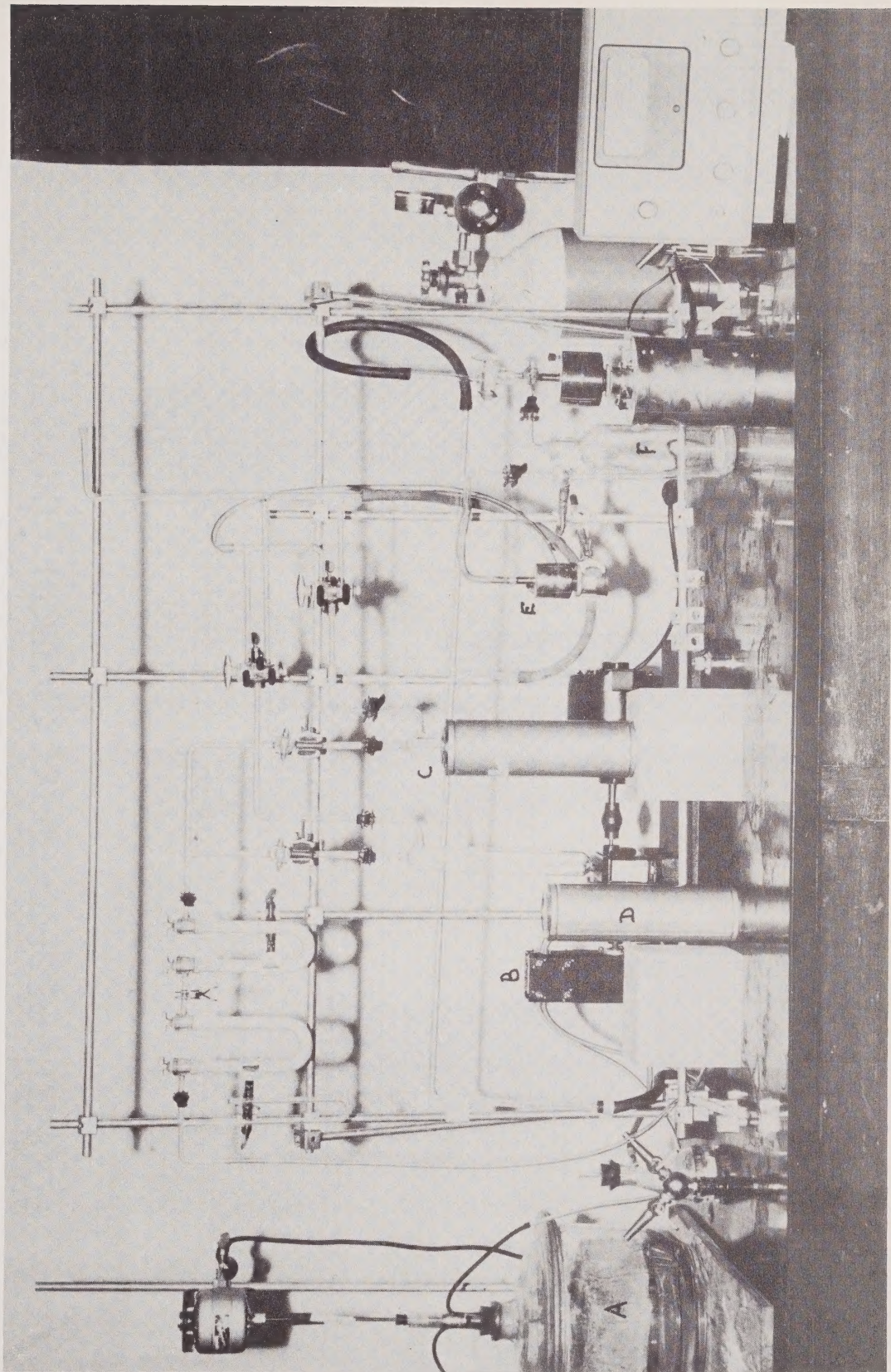


CO_2 from tank A is supplied to chamber E through stopcock C_1 , ball joint D and stopcock C_2 .

By applying vacuum in J, gases are withdrawn from chamber E and dispersed in fritted glass tube G. CO_2 is absorbed in $\text{NaOH } 20\%(\text{H})$. Flask F_2 filled with drierite absorbs water vapor to protect pump.

By alternating stopcock C_1 , in position I and II, the chamber is washed several times with pure CO_2 .

FIGURE 2
APPARATUS FOR CONTINUOUS DETERMINATION OF SPECIFIC ACTIVITY
OF RADIOACTIVE CO_2 IN RESPIRATORY GASES.



$\pm$ SD.
 H N HN

FIGURE 3

PER CENT OF RADIOCARBON INJECTED
 FOUND IN RESPIRATORY CARBON DIOXIDE

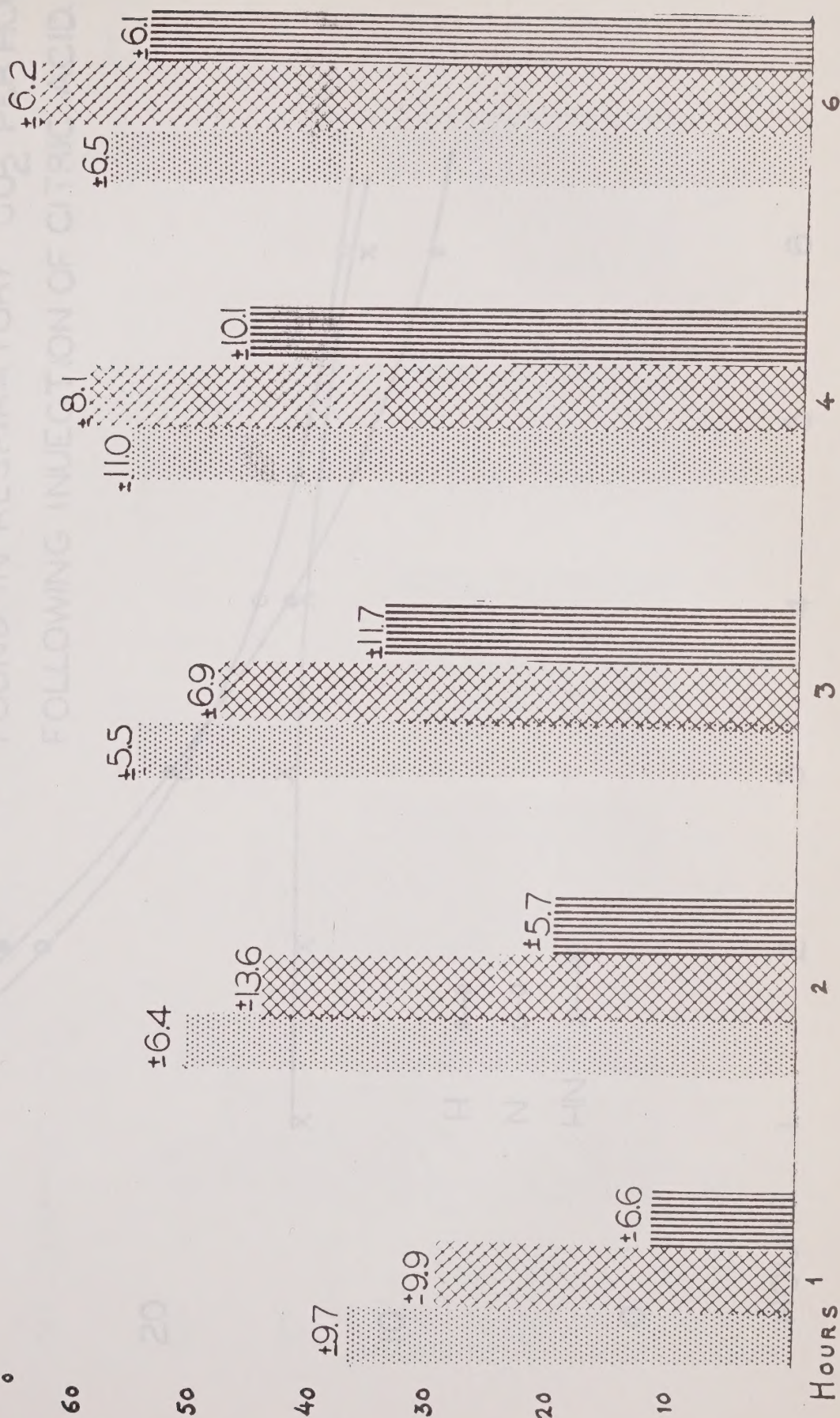
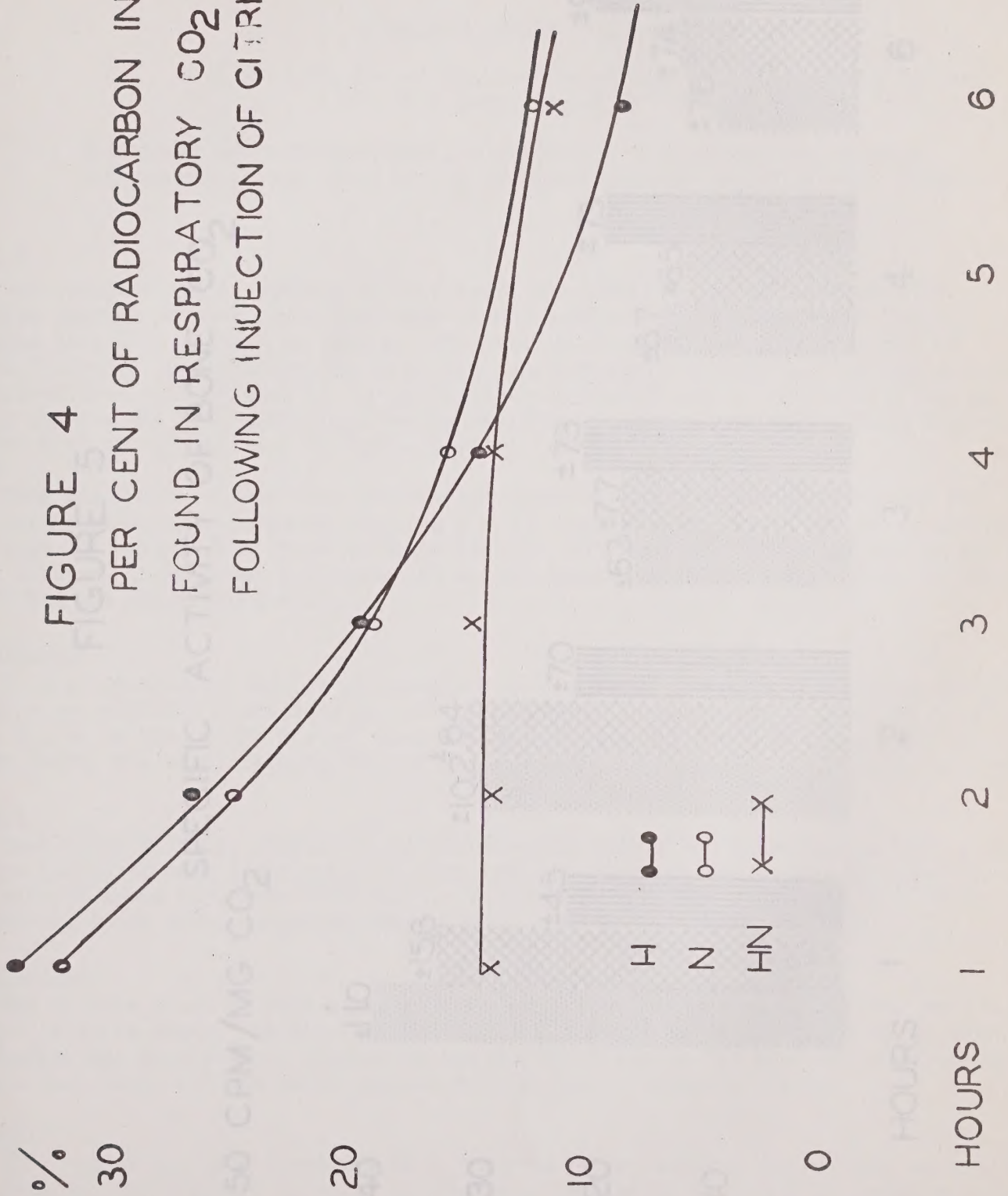


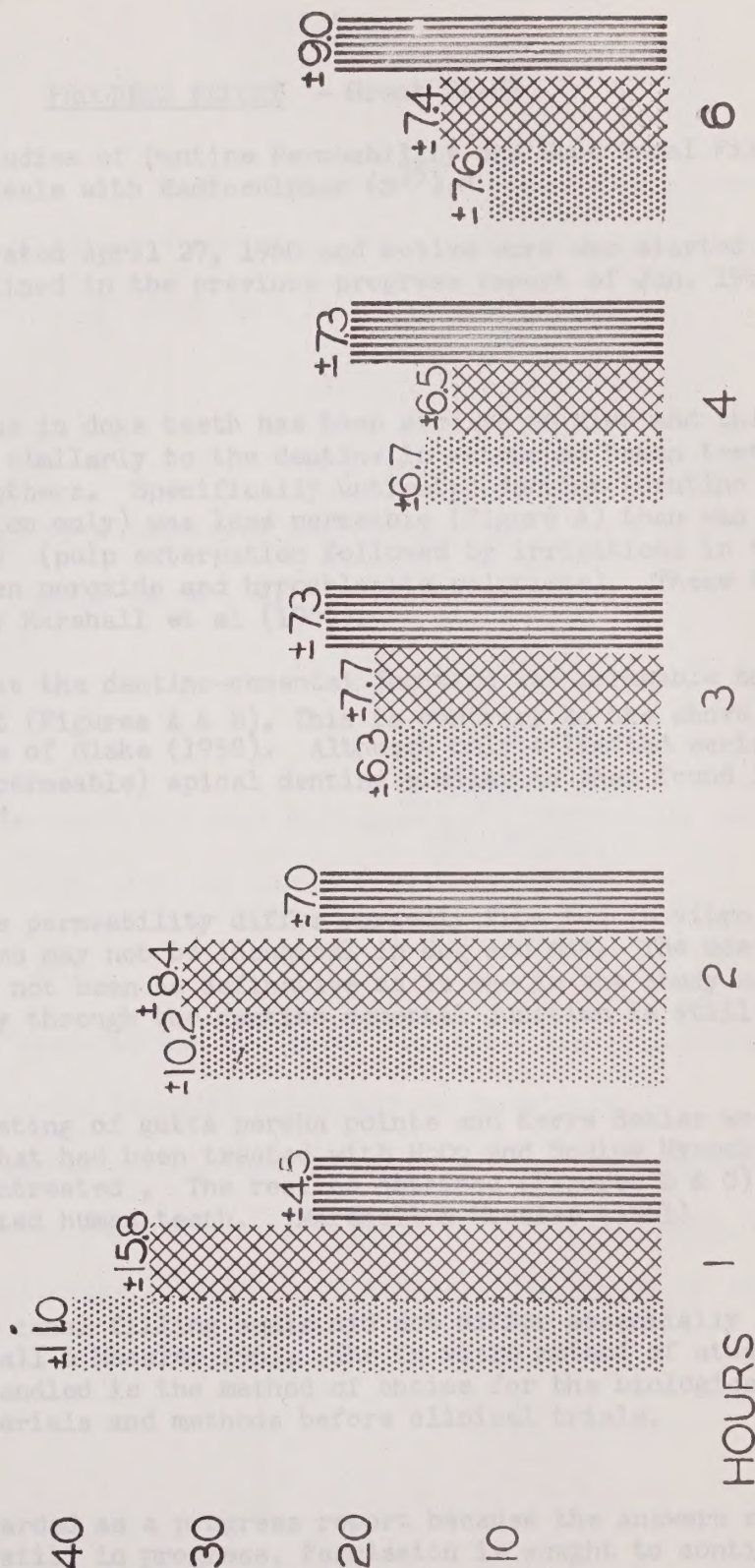
FIGURE 4

PER CENT OF RADIOCARBON INJECTED
FOUND IN RESPIRATORY CO₂ PER HOUR
FOLLOWING INJECTION OF CITRIC ACID.



± SD
H N HN

FIGURE 5
SPECIFIC ACTIVITY OF BONE CO₂
50 CPM/MG CO₂



To: The Associate Committee on Dental Research - National Research Council of Canada.

From: F. James Marshall, Faculty of Dentistry, University of Manitoba, Winnipeg 3, Man.

PROGRESS REPORT - Grant DA-83

"In vivo Studies of Dentine Permeability and Root Canal Filling Seals with Radiosulphur (S^{35})."

This Grant was activated April 27, 1960 and active work was started last summer as explained in the previous progress report of Jan. 1961.

Part A

The permeability of dentine in dogs teeth has been studied in vivo and this dentine reacted in some cases similarly to the dentine in extracted human teeth studied in vitro, and not in others. Specifically untreated dentine (dentine of teeth following pulp extirpation only) was less permeable (Figure A) than was dentine following treatment (Figure B) (pulp extirpation followed by irrigations in the root canal alternately with hydrogen peroxide and hypochlorite solutions). These findings agreed with those published by Marshall et al (1960).

This study also showed that the dentino-cemental junction was permeable to the S^{35} before and after treatment (Figures A & B). This is contrary to the above mentioned findings but agrees with those of Blake (1958). Although only a limited series has yet been studied, transparent (impermeable) apical dentine similar to that found in human teeth has not been encountered.

Conclusions

In vivo studies of dentine permeability differ markedly from the in vitro situation. Dentine permeability mechanisms may not be identical in dog and man. The use of S^{35} as a tracer in this study has not been as definitive as it was in the study of extracted human teeth. The exact pathway through the dentino cemental junction is still in doubt.

Part B

Root canal fillings consisting of gutta percha points and Kerrs Sealer were placed in the canals of dogs teeth that had been treated with H_2O_2 and Sodium Hypochlorite and also in those that were untreated. The results obtained (Figures D & C) were similar to those using extracted human teeth. Marshall & Massler (1961)

Conclusions

The in vivo study of root canal filling seals did not differ essentially from that of the in vitro study. Marshall & Massler 1961. The in vitro method of study which is simpler and more readily handled is the method of choice for the biological assay of all root canal filling materials and methods before clinical trials.

SUMMARY

This report has been forwarded as a progress report because the answers sought were not obtained and the work is still in progress. Permission is sought to continue with this work at least to the extent of the remaining balance of the grant.

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Jour. Dent. Med. Vol. 16, No.4. October 1961.

Blake G.C.

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Proc. Roy. Soc. of Med. 51, 678, 1958.

"Radioisotopes - Special Materials and Services"

published by: Oak Ridge National Laboratory, Oak Ridge, Tenn.

Figure A - Autoradiograph of hemisected dog's canine tooth. S^{35} tracer placed in canal after pulp extirpation only.

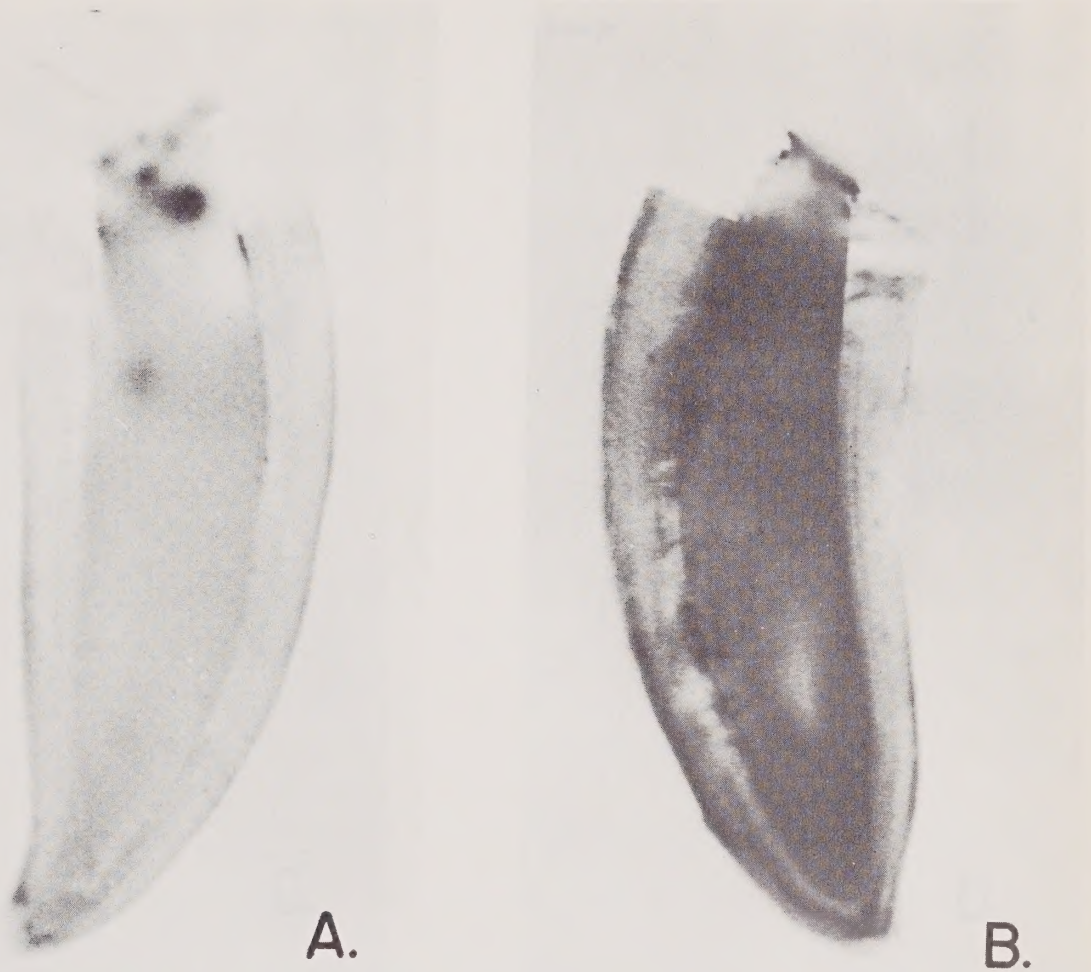


Figure B - Autoradiograph of contra-lateral tooth. S^{35} tracer placed in canal following pulp extirpation and irrigation with H_2O_2 and Sodium hypochlorite solutions. Note increased uptake of S^{35} .

Figure C - Autoradiograph of tooth root filled after pulp extirpation S^{35} tracer placed occlusally after several weeks. Note limitation of penetration along filling margins.



Figure D - Same treatment as in Figure C plus irrigation of canal with H_2O_2 and Sodium hypochlorite after pulp extirpation. Note increased absorption of S^{35} in treated dentine similar to Figure B. Also note lack of marginal penetration.

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

National Research Council

January 1962

Subject: Penetration of minerals from saliva and shifting of minerals in the carious lesion.

Grantee: Z. Mezl.

Project: D.A. 79.

INTRODUCTION:

In caries of the enamel, the minerals are removed from the lesion, or shifted within the lesion, and minerals from outside may enter the lesion.

Three ways of demineralisation are usually considered in the literature: Acid decalcification has been demonstrated and studied in detail. There is less evidence to support the other processes, phosphorylation and chelation, in the carious lesion. The demineralisation is gradual and intermittent and is combined with other processes.

Shifting of minerals from deeper to superficial zones of the lesion is a step in gradual decalcification, but the shifted minerals are not necessarily fixed and visible. The minerals dissolved in a zone undergoing demineralisation may penetrate into deeper parts, or into the enamel around the lesion. Remineralization, by minerals penetrating into the lesion from outside is evident in arrested enamel caries and has been demonstrated in vitro and in vivo, but not as abundantly as demineralization.

REPORT TO THE ASSOCIATE CHIEF OF BUREAU OF DENTISTRY

National Research Council

January 1962

Some of the zones seen in microscopic examinations of carious lesions have been explained by the shifting of minerals. But the existence and properties of certain zones described in the early literature are still a very controversial subject 1,2. Modern techniques allow us to assess the mineral content of a certain area, but it is still very difficult to furnish direct proof that the minerals have been first removed, and then replaced.

The purpose of this work is to study the three types of migration of the minerals in carious lesions, their pathways and their manifestations.

The report is divided into three parts.

1. The microscopic study of carious lesions.
2. The experiments with carious lesions.
3. The lesions produced by acid decalcification in vitro.

Migration and chelation, in the carious lesion. The decalcification is a

slow and intermittent and is associated with other processes.

Shifting of minerals from deeper to superficial zones

of the lesion is a step in gradual decalcification, but the shifted min-

erals are not necessarily fixed and visible. The minerals dissolved in a

zone undergoing decalcification may penetrate into deeper parts, or into

the enamel around the lesion. Remineralization of minerals penetrating

into the lesion from outside is evident in arrested enamel caries and has

been demonstrated in vitro and in vivo, but not as a mechanism of dentin

formation.

1- MICROSCOPIC STUDY OF CARIOUS LESIONS

Microscopic observation may lead to important conclusions on the migration of minerals in the carious lesion but only if the deposition of minerals along with their fine structural details can be shown. This should be achieved by a technique which does not result in artifacts and which can be applied to material in large numbers.

Materials and Techniques:

Initial carious lesions without loss of substance were studied on surfaces ground perpendicularly to the enamel rods and seen in reflected light.

Most of them were lesions on a smooth surface. With the naked eye one can distinguish white spots, yellow-brown spots; and arrested caries characterized by dark brown to black spots with a hard smooth surface or interproximal attrition.

The ground surfaces were prepared by hand or on a polisher using diamond-flex paper no 600 A, emery polishing paper no 4/0 and gamal polishing cloth with alumina. In some lesions serial surfaces were progressively prepared and observed. Using oblique reflected light in an ultropak microscope no etching nor staining is necessary. When the rods are seen in cross-sections, details in the interior of the rod, the rod sheath and the interprismatic substance can be distinguished better than in other microscopic techniques.

Results:

Until now, areas with the following aspect have been observed:

- 1.- White cross sections of rods were seen on the dark background of the interprismatic substance. Transitional forms were also noted: white wide rod sheath and rod periphery on dark background, or grey rod cross sections on dark background.
- 2.- Homogeneous white highly reflecting areas.
- 3.- Thin white rod sheaths, dark peripheries and white cores of the rods on dark background.
- 4.- Dark rod cross sections on white background and transitional forms such as: rods with dark peripheries and white centers on white background.
- 5.- Homogeneous, cloudy, grey, semitranslucent areas with transitions to a translucent, dark or pigmented aspect.
- 6.- Semitranslucent, cloudy, finely granulated areas with slightly visible greish rod cross sections.

Table one shows how many times each of these alterations have been seen in the three main types of lesions in the first 300 cases examined in this project.

TABLE 1

MICROSCOPIC ALTERATIONS FOUND IN CARIOUS LESIONS.

	<u>Number of observations in</u>		
	White lesions	Brown lesions	Arrested Caries
White rods, dark background	128	42	16
White area	131	63	37
White cores, dark background	21	19	13
Dark rods, white background	40	39	17
Semitranslucent, homogeneous	33	51	52
Semitranslucent, cloudy structure	99	70	54
Number of lesions	156	77	67

In white lesions, the white areas and white rods predominated in frequency and in extent. The homogeneous white area often forms the major part of the lesion. 40 lesions contained only white areas and white rods, 9 consisted exclusively of white rods; 5 of these 9 were very small, and were invisible with the naked eye.

In brown lesions and arrested caries, the semi-translucent areas with a cloudy structure, and the semitranslucent to translucent areas without visible structure predominated in frequency and in extent. In arrested caries, 33 lesions contained only the semi-translucent or homogeneous translucent areas.

The white substance is usually in the center of the lesions. The homogeneous semitranslucent substance forms often a thin layer at the surface of the enamel, corresponding to the superficial zone known from longitudinal sections. Otherwise, the alterations do not appear in any regular disposition which could be considered as characteristic zone formation. In cross sections, most carious lesions are much more irregular than they appear in longitudinal sections.

The hardness of the observed areas.

The review of the pertinent literature ³ shows considerable variation in the hardness of enamel in different teeth, and in different areas of enamel in the same tooth. These variations have been ascribed to changes in the composition of the enamel, and to differences in the direction in which the hardness test was made with respect to the orientation of the crystals. The degree of hardness is not necessarily proportional to the degree of mineralization or demineralization of the enamel. With respect to these facts, and to the fact that the depth

of the observed surfaces varied, only quite marked differences in the hardness were considered of value for this work.

Materials and techniques:

The width of exact straight scratches made on the observed surface by a diamond indenter with a constant load was measured at selected points. From five to ten measurements, in microns, were made in each microscopically characteristic area. Small areas were not considered.

The hardness varies inversely with the size of the indentation when the load is constant and relative hardness numbers ⁴ could be computed from the measurements. The scratch method has been used in this work for comparing the softening of various areas in a lesion, but it is not suitable for computation of absolute hardness values.

Results:

The results from the first 200 lesions tested were classified according to the microscopically characteristic areas, and graded in three groups. Table II, shows for each characteristic area, the numbers of cases in which the highest measurement was not more than 11 microns, from 12 to 19 microns, and finally 20 microns or more. Measurements in normal enamel are usually 10 microns, (± 1 micron). At 40 microns the material is splintered and squashed.

TABLE IIMEASUREMENTS IN MICROSCOPICALLY CHARACTERISTIC AREAS

	<u>Number of Cases</u>		
	Up to 11 microns	12 to 19 microns	20 microns & over
White rods, dark background	2	61	20
White area			162
White cores dark background	4	1	
Dark rods white background	13	4	
Semitranslucent homogeneous	115	9	
Semitranslucent, cloudy structure	53	20	1

As is to be expected, white areas are soft and demineralized. Semitranslucent to translucent areas without visible organic structure, are as hard as normal enamel, i.e. mineralized. The readings from 12 to 19 microns compared, on the same ground surface, with 10 of normal enamel indicate the gradual softening of the transitional areas.

II. EXPERIMENTS WITH CARIOUS LESIONS

The penetration of liquids into the initial carious lesions of the enamel was studied by exposing the teeth to various solutions. The penetration depends on the solution used, on the length of time the tooth is immersed and on the type of lesion and on characteristics of the tooth.

Material and techniques.

In previous experiments, various staining and mineral solutions and techniques were tried.

Most of the studies were done following the submersion of the teeth in the following solutions: A solution of 1% toluidine blue in distilled water. A solution of 0.02% calcium hydrogen orthophosphate labeled with P^{32} in distilled water, 1 mc in 1000 ccm.

The time varied from 2 hours to several days. Of greatest interest were the observations made after submersions of 2 to 6 hours.

The characteristics of the lesions (especially the depth) and the properties of the teeth (presence of lamellae, cracks etc.) varied considerably and could not be assessed before the experiment. Consequently, some cases were difficult to interpret.

The penetration of stains was visible with the naked eye, and microscopically, in ground sections, or on ground and polished surfaces seen in reflected light. The latter technique was the one mainly used. Some teeth had been cut longitudinally through the lesion; in other teeth, the surfaces were ground perpendicularly to the rods and various depths of

the lesion were examined. Cutting and polishing were done using a minimal amount of water in order to avoid spreading the stain.

The penetration of the phosphate into the lesions was visualized by autoradiography. After submersion, the teeth were cut in serial sections on a hard tissue microtome. The sections were glued on paper and placed in contact with a photographic emulsion. After an exposure of 16 hours using Kodak Tri-X film, or 4 days using Kodak medium lantern slide plates, the autoradiographs were developed. The sections were polished and mounted in distilled water. They were studied in polarized light and compared with their autoradiographs.

Results:

A. The penetration of a solution of toluidine blue into initial carious lesions during periods ranging from 2 to 5 hours was studied on 143 teeth with 200 lesions.

Following submersion in toluidin blue, some lesions were stained and some were not stained at all, and some lesions were partially stained⁵. The results are shown in table III.

TABLE III

CARIOUS LESIONS SUBMERGED IN TOLUIDIN BLUE

	White lesions	Brown lesions
Not stained	18	69
Stained	49	27
Partially Stained	17	20
Total Number	84	116

1. Among the lesions which did not stain, 56 have been examined in longitudinal sections. In all cases, the superficial zone of the lesion was translucent and mineralized.

In 27 cases, the surface of the lesion was lightly ground and polished and examined. No stain was evident in the deeper parts of the lesion. The tooth was submerged for a second time into the staining solution but for a much shorter time, in most cases for 3 or 4 minutes. In all cases, the lesion was stained following this second submersion, except the translucent hard areas.

In 15 cases, the lesions which did not stain, were submerged for 2 hours in lactate buffer solution at pH 5.12 at 37° C, and then submerged again in toluidin blue for 2 hours. Following this all of the lesions were completely stained.

2. From the lesions which were stained following the initial submersion 58 have been examined in longitudinal sections. In 54 cases, no particular zone was seen at the surface of the lesion, in 2 cases, the superficial zone of the lesion was translucent, in 2 the superficial zone was either thin or faintly translucent or not continuous. In 18 cases the surface was ground and polished and the lesion studied at various depths. All these lesions were deeply stained.

3. From the lesions which stained partially, 19 have been examined on longitudinal sections. In 8 cases, there was a superficial translucent zone in the part of the lesion which did not stain and there was no such zone in the part which was stained.

B. The penetration of a solution of calcium phosphate into the initial carious lesions was studied in 92 teeth with 145 carious lesions ⁵. In 42 cases, the phosphate did not penetrate into the lesion in a quantity which could be shown on the autoradiographs. Even in the 8 cases where the teeth had been submerged for four days, the lesions were not visible on the autoradiographs. In all these 42 lesions the examination of the ground sections in polarized light reveals a well mineralized superficial zone.

In the remaining 103 cases, the phosphate penetrated into the lesions in a quantity sufficient to produce a dark triangle (Fig. 1). on the autoradiographs. Most of these teeth were submerged for four hours. Ground sections of these lesions were examined in polarized light and showed, in 4 cases a mineralized superficial zone, in 70 cases there was no particular zone at the surface, in 29 cases the superficial zone was interrupted or the degree of mineralization was low.

C. The uptake of D32 in ground sections.

In these cases, where the autoradiographs did not show any penetration of the phosphate into the carious lesion after submersion of the whole tooth in the solution for four hours, a second experiment was done: The ground sections were submerged in the solution for 1 hour and autoradiographs were again made.

On the second autoradiographs, all lesions show a high uptake of P 32. (Fig. 2).

This part of the project has not yet been concluded.

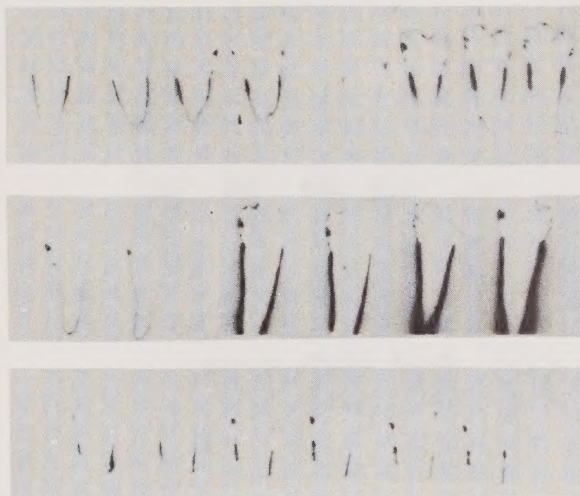


Fig. 1. - Proximal carious lesions visible as dark triangles on the autoradiographs of the teeth.

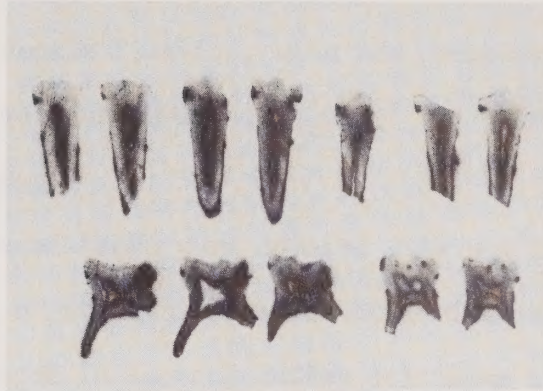


Fig. 2. - All lesions are dark on the autoradiographs after submersion of ground sections in the solution containing P 32.

III. LESIONS PRODUCED BY ACID DECALCIFICATION IN VITRO

Artificial lesions have been produced in vitro⁶ on normal enamel by acid solutions to compare the manifestations of pure and continuous acid decalcification, with the phenomena seen in polished surfaces of natural carious lesions.

Material and techniques.

Selected teeth were covered with wax leaving a small window on the normal enamel of the caries resistant labial and lingual surfaces. Then they were submerged in lactate buffer solutions of a pH 5.12 and 4.93 for periods from 50 hours to 7 days, and in acetate buffer solutions of a pH 4.0 and 4.5 for periods from 20 hours to 12 days.

Some of the teeth with artificial lesions have been studied on longitudinal ground sections embedded in Canada balsam, distilled water, solution of mercuric iodide and potassium iodide R.I. 1.62. The ground sections have been examined with polarized light. Some of the lesions have been studied on ground and polished surfaces seen in reflected light. Some of the teeth have been studied after submersion in toluidin blue and others after submersion in radioactive phosphate.

Results:

Acid decalcification produced three types of lesions in the enamel:

1. Some lesions showed a gradual loss of minerals increasing from the deeper parts to the surface, when examined in ground sections seen in polarized light.

- Teeth with artificial lesions were submerged for 4 hours in calcium phosphate solution labelled with P 32. The autoradiographs of all these teeth showed large dark spots even in areas of very shallow lesions, indicating a high degree of demineralization.
2. Some lesions showed a loss of minerals in the interior while the superficial zone contained more minerals than the deeper parts of the lesion.
 3. Other lesions showed a gradual loss of minerals and the beginning of cavity formation. Lesions of this type were not used in the experiments involving the penetration of solutions.

When examined in reflected light using the same techniques as were used in studying carious lesions, the artificial lesions were conspicuous by their uniformity.

All contained large, white very brittle areas and white rods. Areas with white rods sometimes formed a very narrow border thus indicating a rapid transition from normal to highly decalcified enamel.

Some artificial lesions contained areas characterized by a particular widening of the rod sheaths described by Lemieux⁶, which are very rare in carious lesions.

Other phenomena which were seen in small areas of a few lesions were: small, semitranslucent cloudy zones at the surface; a few rods with dark periphery and white cores.

Homogeneous translucent areas have not been seen in the artificial lesions examined until now.

Teeth with 31 lesions produced by lactate buffer, and 19 lesions produced by acetate buffer were submerged for three hours in toluidin blue. All 50 lesions stained intensely, even the lesions with the superficial zone containing more minerals than the deeper parts of the lesion.

Teeth with arteficial lesions were submerged for 4 hours in calcium phosphate solution labelled with P 32. The autoradiographs of all these teeth showed large dark spots even in cases of very shallow lesions, indicating a high uptake of phosphate. (Fig. 3).

This part of the project is not as yet completed.

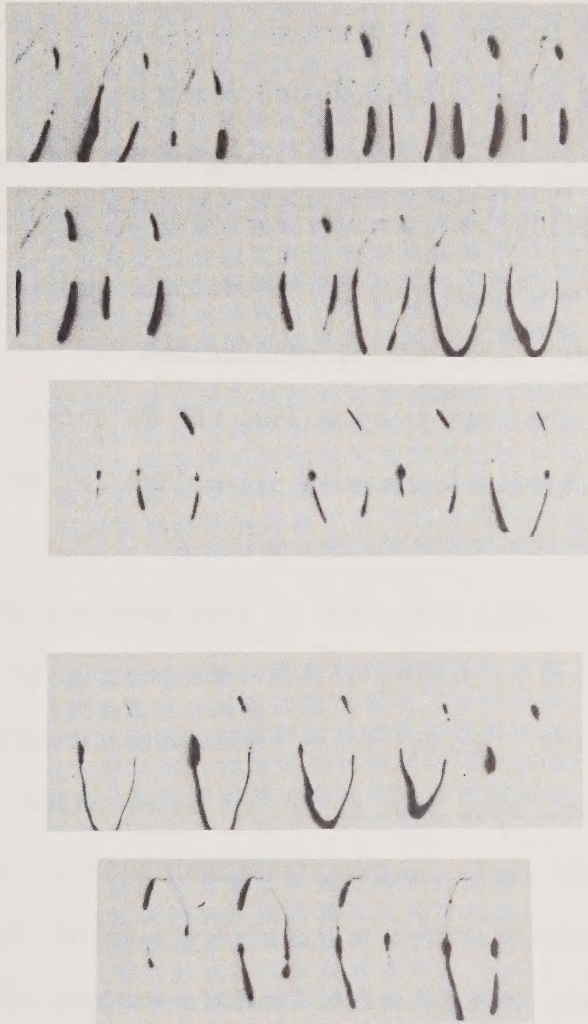


Fig. 3. - Shallow lesions produced by lactate buffer (top) and acetate buffer (bottom) produce a wide dark zone on the autoradiographs of the teeth.

DISCUSSION

The alterations observed in initial carious lesions of the enamel on ground surfaces cut perpendicularly to the rods have been described and discussed in previous reports ⁷.

Since the previous report, two publications have appeared ^{8,9} based on observations of microradiographs of ground sections of initial carious lesions, cut perpendicularly to the rods. They show that carious demineralization begins at the periphery of the rod, then involves the prism body, leaving the interprismatic substance largely undemineralized until last.

On surfaces seen in reflected light, white rods and white areas predominate in frequency and in extent in white lesions which are according to clinical experience acute caries. White areas are soft, i.e. demineralized. White areas and white rods were found in all artificial lesions produced by acid demineralization. This indicates that this microscopic aspect is characteristic of active demineralization.

The semitranslucent to translucent areas predominate in frequency and in extent in brown lesions which are usually considered to be chronic and in lesions of arrested caries. This suggests that they are a microscopic manifestation of a slow or a healing process. They are hard, i.e. mineralized; this can be confirmed with polarized light in the superficial zone ¹⁰.

Homogeneous translucent areas have not been seen in artificial lesions. Semitranslucent cloudy areas have been seen in artificial lesions but infrequently, and to a lesser extent.

In artificial lesions there was no remineralisation from the outside and the possibilities of shifting of minerals are very limited.

This suggests that the phenomena which were absent in the artificial lesions, but present in caries, and frequently present in arrested caries, resulted from the penetration of minerals from the saliva and the shifting of minerals in the carious lesion.

The purpose of these experiments with carious lesions was to find out whether there were some differences in the properties of the initial carious ^{lesions} and whether they could be correlated with the various aspects of the lesions. The differences in the quantity of solution which penetrates into the lesions under the conditions used during these experiments might be of practical importance.

(It is probable that modified techniques or more delicate methods of assessment might show some penetration in all lesions, but this was not the purpose of this study).

The mineralized superficial zone was present in all lesions in which the solutions did not penetrate, and in a few lesions into which the solutions did penetrate.

This suggests that a well developed superficial zone of certain quality did not allow the penetration of the solutions into the lesion. This might be an important factor in the evolution of the lesion.

In many arteficial lesions, the superficial zone was more mineralized than the deeper parts of the lesion, and this zone was relatively thick while the lesions were shallow. Nevertheless, all of the arteficial lesions examined until now stained well and their autoradiographs

showed that the P_{32} penetrated well into the lesion.

This suggests that the penetration of minerals from saliva might be the factor which makes the superficial zone in certain carious lesions impermeable.

The superficial mineralized zones, even when they are of a similar aspect, are not all of the same character.

The formation and the properties of the superficial mineralized zone and its role in the evolution of the carious lesion merits further investigation.

CONCLUSIONS

Examination of transverse rod cross sections shows the distribution of minerals in the enamel with fine structural details and allows certain conclusions as to the migration of minerals in carious lesions.

The alterations found by this technique in carious lesions have been described.

The alterations predominant in white carious lesions and in arteficial lesions produced by acid decalcification, are considered to be manifestations of active demineralization. The alterations predominant in brown lesions and arrested caries and absent in arteficial lesions, are considered to be manifestations of the shifting of minerals and the penetration of minerals from saliva into the carious lesions.

Under the conditions used during these experiments the phosphate solution did not penetrate in an appreciable quantity into certain carious lesions; in these lesions the superficial zone was more mineralized than the deeper parts of the lesion.

Certain superficial zones in carious lesions and all superficial zones in arteficial lesions appeared to be permeable under the conditions used during these experiments.

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APPENDIX L

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

NATIONAL RESEARCH COUNCIL

January 1962.

Subject: The Relation of Nutrition to Morphology and Caries Susceptibility
in the Teeth of Rats.

Grantee: K.J. Paynter and A.M. Hunt

Project: DT 72

Amount of Grant: Three year term grant
\$5,000. per year.

Of the information that has been obtained from this study during the past three years, probably the most significant is (a) that in indicating the great importance of vitamin D in the diet during formation on caries susceptibility, and (b) that pertaining to the mechanism by which factors act during tooth development to change form and susceptibility. For the sake of convenience these will be discussed separately.

A. Nutrition, Tooth Form, and Caries Susceptibility.

Corroboration for the basic concept that has emanated from this study, (Paynter and Grainger, 1956)-that tooth form could be altered during development by experimental means,-has now been obtained from other sources. Shaw (1960) has shown that low protein diets result in smaller and more caries-susceptible teeth, and more recently McMurchy (1961) has reported somewhat similar affects in the hamster using high phosphate diets. Our own data, some obtained by design and some by accident, continues to support this concept as well.

While continuing with the studies outlined in the report to the Committee in 1959 (Proc. 25th meeting, Feb./59 App.H) the experimental animals were moved from quarters well-illuminated by sunlight in the old dental building to new quarters in the new dental building in Toronto.

The latter, although infinitely more adequate, were designed to almost exclude sunlight. This resulted in animals on the synthetic diets (including the control) subsequently developing bone changes indicative of rickets. In the old quarters it had been unnecessary to include vitamin D in the diet since the animals were quite able to synthesize adequate amounts. This was not so after the move into new quarters.

The effect on the caries susceptibility of teeth developed before and after the move was dramatic, and it was possible when evaluating caries experience in offspring in the control group (on a "complete" synthetic diet to determine from caries alone whether the teeth were developed before or after placing the mothers in the unlit rooms. Those developed in old quarters remained relatively resistant to decay; those developed in the new building were very much more susceptible.

While this turn of events was of considerable interest, and of help in further experimental design, nevertheless lack of detailed records of animals in relation to the time of pregnancy and the move, and the unknown effects on the experimental groups, rendered the data gathered from these animals of relatively little value, with the exception of that from the group on the diet with the high phosphorus/calcium ratio where the increased caries susceptibility was evident and valid (Paynter and Grainger, 1961).

To control this apparently highly sensitive Vitamin D effect for subsequent experiments the animal rooms have been blacked out so that no daylight is permitted to enter at all. The vitamin D intake can thus be controlled much better through the diet.

Two long-term nutritional studies have begun, -(1) to test the effects of fluoride and vitamin D on tooth morphology and caries susceptibility, and their interaction, and (2) to test the effect of various calcium and phosphorus levels, and their interaction, both with and without vitamin D in the diet.

1. Fluoride and vitamin D.

In view of this readily demonstrated and important relation between vitamin D and caries demonstrated in the rats, and shown in the past in humans (Anderson et al, 1934; Mills, 1937), and in view of the fact that McHenry (1961) has recently reported that in Stratford, Ontario about three fourths of a group of children studied for nutritional intake had little or no vitamin D in their diets, it seemed worth while to study further the effects of this vitamin on caries in a controlled experiment, and to try to determine its interaction with fluoride intake in relation to caries susceptibility. An experiment was designed for this purpose as follows:

	Fluoride*	No Fluoride
Vitamin D**	Fluoride Vitamin D	No Fluoride Vitamin D
No Vitamin D	Fluoride No Vitamin D	No Fluoride No Vitamin D

*Fluoride 3 ppm. in drinking water.

**3 international units per gram of diet.

Animals were treated as previously described, i.e. mothers were kept on the experimental diet through pregnancy and lactation (and on D -deficient diets for three weeks before mating). At weaning the young were anaesthetized and the upper right first molar extracted for study morphologically, and the animals maintained for 120 days on a cariogenic diet. Examination for caries was made following this period.

Some of the crude data which has been obtained is shown in Table I.

TABLE I

Mean group tooth size and shape measurements, and caries scores for rats on diets varying in content of vitamin D and fluoride.

+ D + Fl			+ D - Fl		
F o r m	M.D. Diam.	88.9 mm	F o r m	M.D. Diam.	89.8
	M.D. Cerv.	78.1 mm		M.D. Cerv.	79.4
	Fiss. Angle	6.7 °		Fiss. Angle	9.3
C a r i e s	No. Teeth	3.07	C a r i e s	No. Teeth	3.15
	No. Cavities	3.07		No. Cavities	3.17
	Extent "	5.42		Extent "	4.90
- D + Fl			- D - Fl		
F o r m	M.D. Diam.	87.7	F o r m	M.D. Diam.	88.7
	M.D. Cerv.	76.7		M.D. Cerv.	78.5
	Fiss. Angle	8.4		Fiss. Angle	7.6
C a r i e s	No. Teeth	4.00	C a r i e s	No. Teeth	4.80
	No. Cavities	4.55		No. Cavities	5.52
	Extent "	9.15		Extent "	8.44

The detailed statistical analysis of the data has not yet been made. However even from the figure presented it seems evident that morphological results confirm previous findings, i.e. fluoride supplementation and vitamin D deficiency both have a tendency to reduce tooth size. The fluoride effect on caries is evident although not as dramatically as expected, which may relate to the

relatively low dosage employed. The most dramatic caries effect is that related to vitamin D. Deficiency of this vitamin during tooth development results not only in an increase in number of cavities, but also a large increase in cavity size extent. Whether this reflects more rapid progress of the lesion through tooth substance or an earlier attack, and therefore more spread because of increased time during which the lesions could spread, cannot be determined by these data.

In the near future the data will be analysed, interaction calculated, and related to animal growth, parental tooth size, etc.

b) Calcium and Phosphorus.

In rats the calcium and phosphorus intake and proportions in the diet during tooth development has been of interest for some time. In the early studies on this subject, (Paynter and Grainger, 1956), phosphate was raised in the diet to tip the P/Ca ratio to 3/1, in order to simulate a vitamin D deficiency with subsequent dental morphology and caries susceptibility changes. McMurchy (1961) has recently reported somewhat similar effects on tooth morphology in the hamster by high phosphate feeding.

To clarify these relationships, and the influence of vitamin D on them, experiments were designed as follows:

		Calcium %			
		0.15	0.5	1.0	
Phosphorus %	0.15	Low Ca. Low P.	Norm. Ca. Low P.	High Ca. Low P.	(a) with vitamin D present.
		Low Ca. Norm. P.	Norm. Ca. Norm. P.	High Ca. Norm. P.	(b) with vitamin D absent.
	0.5	Low Ca. Norm. P.	Norm. Ca. Norm. P.	High Ca. Norm. P.	
		Low Ca. High P.	Norm. Ca. High P.	High Ca. High P.	
	1.0	Low Ca. High P.	Norm. Ca. High P.	High Ca. High P.	
		Low Ca. High P.	Norm. Ca. High P.	High Ca. High P.	

The first experiment is now well under way, and tooth morphology data are now available for several of the cells. Animals from the same cells are now on the caries-producing regime. More difficulty is anticipated in obtaining data from the second experiment where animals are to be kept on a vitamin D-deficient diet.

B. Mechanism of Action of Factors Affecting Tooth Form and Caries Susceptibility.

Our own studies and others (Shaw, 1960; McMurchy, 1961) have confirmed that tooth form is subject to alteration during development, and that sometimes these alterations are associated with changes in caries susceptibility, and sometimes they are not. The pattern of susceptibility has not been predictable in advance from the tooth form alterations. For example, diets low in vitamin A or protein, or high in phosphate or fluoride (12 ppm) result in teeth smaller than normal, although the pattern of caries susceptibility varies considerably. It is unchanged with vitamin A deficiency, lower with fluoride supplementation, and higher with high phosphate feeding or a low protein diet. In order to understand the processes involved it was recognized that further knowledge would have to be obtained as to the manner in which these teeth develop. Information has been obtained using two methods, - reconstructions of developing tooth germs at various ages, and autoradiographs of developing teeth after injection of animals with tritium-labelled thymidine, to follow mitotic activity.

(a) Reconstructions.

Sections, 12 microns thick, were cut of developing upper first molar teeth of rats at various ages from 19 days in utero to 10 days after birth. Every second section was projected onto a sheet of plastic one-sixteenth inch in thickness at a magnification of sixty-six times. This combination of

section and plastic thickness, and magnification, permitted the construction of models unchanged in proportion from the original. The age of animals and the number of specimens is shown below;

<u>Age.</u>	<u>Number of specimens.</u>
19 days in utero	1
20 days in utero	1
Birth	2
1 day postnatal	2
2 days "	2
3 " "	1
4 " "	1
7 " "	1
10 " "	1

The plastic was cut along the dentino-enamel junction so that two models were made of each tooth germ,- one of the dental papilla including dentin where present, and the other of the enamel organ including enamel where present.

Measurement made of the models at the dentino-enamel junction included mesio-distal and bucco-lingual diameter, crown height, the length of the sides of the grooves, the distance from cusp tip to cusp tip, and the angle between the cusps and the base of the crown. The measurements obtained are shown below in Table II.

Table II

Measurements at the Dentino-enamel Junction of Reconstructions of Developing Upper First Molar Teeth in the Rat.
(magnification factor- 66)

Age	Crown Size			Distance Cusp-Cusp		Cusp Angle			Length Groove Sides			
	M-D	B-L	Ht	Me-Ce	Ce-Di	Di	Ce	Me	Dist. Gr.	Mes. Gr.		
19 in	33.2	12.1	6.7									
20 d	66.0	30.2	29.2	23.2	29.2							
B(A)r	136.7	57.5	55.5	49.5	55.0	20.5°	47.0°	53.0°	66.5	23.5	59.7	22.2
(b)	157.0	73.5	72.0	57.1	59.5	24.0	43.0	60.0	75.0	36.0	70.0	29.0
1 (a)	140.5	60.5	64.0	43.5	64.0	21.0	52.0	64.0	72.0	29.0	61.5	30.5
(b)	149.5	66.5	71.5	50.7	53.0	22.5	54.5	60.5	69.5	30.0	60.5	32.0
2 (a)	137.5	60.0	59.5	46.0	55.5	26.0	55.5	61.5	63.0	34.0	61.5	31.5
(b)	164.0	91.0	77.5	47.0	64.5	41.5	67.5	64.5	75.0	43.5	73.0	39.5
3	173.0	38.7	91.2	56.0	60.0	62.0	63.5	66.0	70.0	44.0	73.5	54.0
4	136.5	87.5	102.0	56.5	63.5	60.5	69.0	70.5	63.0	47.5	63.5	43.0
7	205.0	123.0	101.5	64.5	63.0	66.5	73.5	74.5	73.5	46.0	76.5	50.0
10	217.0	113.7	113.0	64.7	63.5	63.0	73.5	77.0	96.5	60.5	97.5	63.0

b) Autoradiographs.

Rats were injected one or more times intraperitoneally with tritium-labelled thymidine at a concentration of from 1-2 microcuries per gram of body weight, and sacrificed at various times after injection as shown in Table III below:

Table III

Number of Rats, Age, and Schedule of Tritium-Labelled Thymidine Injections for Study of Molar Tooth Development.

No. Animals	No. Injections	Age At Injection	Number of Specimens, and Time of Sacrifice.
8	1 per animal	Birth	2 each at 1 and 5 hours 1 and 10 days
6	1 per animal	1 day	2 each at 1 and 5 hours 1 day
6	1 per animal	2 day	2 each at 1 and 5 hours 1 day
5	1 per animal	3 day	1 each at 15 mins. 1 and 4 hours 6 and 10 days
6	2 per animal	3 & 6 day	1 each at 15 mins. after 2nd 1 and 4 hours after 2nd 10,20,40 days after 1st
5	3 per animal	3,6, & 10 days	1 each at 15 mins. after 3rd 1 and 4 hours after 3rd 20 and 40 days after 1st
15	1 per animal	14 day	2 each at 1,2, and 3 hours 1,2,4,7, days 1 at 25 days

Observations

a) Reconstructions.

It was quite obvious from the study of the reconstructed tooth terms, that the state of development of the tooth varies widely in relation to the age of the animal. The molar tooth from one animal at birth (Table II, #B(b)) was more advanced developmentally over other specimens even two days old. This one was even further developed than its own littermates at older ages, indicating variation even within litters at the same age.

However in spite of the wide variations, it was possible to visualize from the data the changes that occur as the crown develops and attains final form. Figure 1 shows a graph of the mesio-distal diameter of the crowns plotted against age.

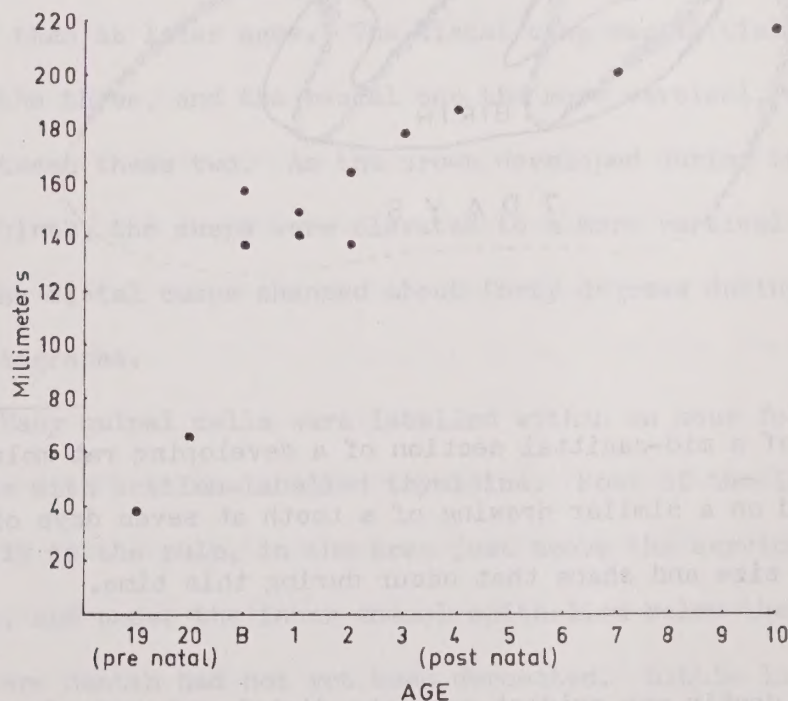


Figure 1. Graph showing the increase in mesio-distal diameter of the dental papilla (including dentin) of developing upper first molars in the rat, from 19 days in utero to 10 days postnatal. Measurements are approximately sixty-six times actual size.

The same data platted on log paper, indicated that until at least ten days, tooth size is a factor of the log of age. Similar results were obtained with bucco-lingual diameter and crown height. The mesio-distal diameter quadrupled in size during the two days before birth, and continued to grow after birth so that the final diameter was about one and one-half times that at birth. Crown height continued to increase relatively rapidly over a longer time than width or length, as the crown continued to grow vertically after the other measurements were more or less established. It was during the period of rapid growth just before birth, that the cusps became apparent on the models.

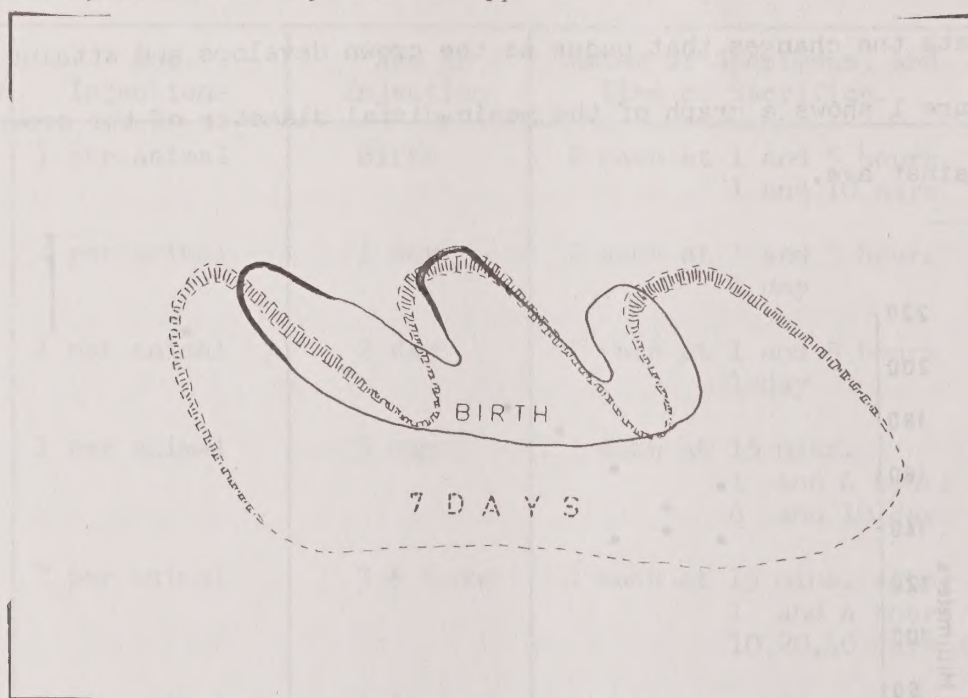


Figure 2. Drawing of a mid-sagittal section of a developing rat molar at birth, superimposed on a similar drawing of a tooth at seven days of age, to show changes in size and shape that occur during this time.

At birth dentin was evident over the distal and central cusps, and by two days of age, it was also present in the mesial cusp. Dentin apposition spread mesio-distally and laterally from the cusp tips, and by three or four days, it had joined the cusps together under the base of the grooves.

No enamel was evident in any tooth until three days, and then its pattern of appearance and deposition closely paralleled that for dentin.

The tips of the cusps moved apart from one another relatively rapidly until dentin apposition began, at which time the intercuspal distances became relatively stable (Table II). Whether some further separation occurred as dentin was deposited down the sides of the cusps could not be determined for certain from the present data.

The groove sides continued to lengthen until dentin was deposited under the base of the fissures, after which no further increase in groove depth occurred (Table II). The distal slope of each groove increased both absolutely and relatively more than the mesial slope. Some idea of the magnitude of this increase can be obtained from figures 2 and 3.

In the beginning the cusps developed with their long axis more horizontal than at later ages. The distal cusp was initially the more horizontal of the three, and the mesial one the more vertical, with the central cusp falling between these two. As the crown developed during the three days following birth, the cusps were elevated to a more vertical position, and the angle of the distal cusps changed about forty degrees during this period.

b) Autoradiographs.

Many pulpal cells were labelled within an hour following injection of the rats with tritium-labelled thymidine. Most of the label was found peripherally in the pulp, in the area just above the cervical loop and Sheath of Hertwig, and under the inner enamel epithelium below the base of those grooves where dentin had not yet been deposited. Little labelling occurred in the central part of the pulp, and most of the cells that were labelled in this site, were related to vascular proliferation into the area (Figure 3.).

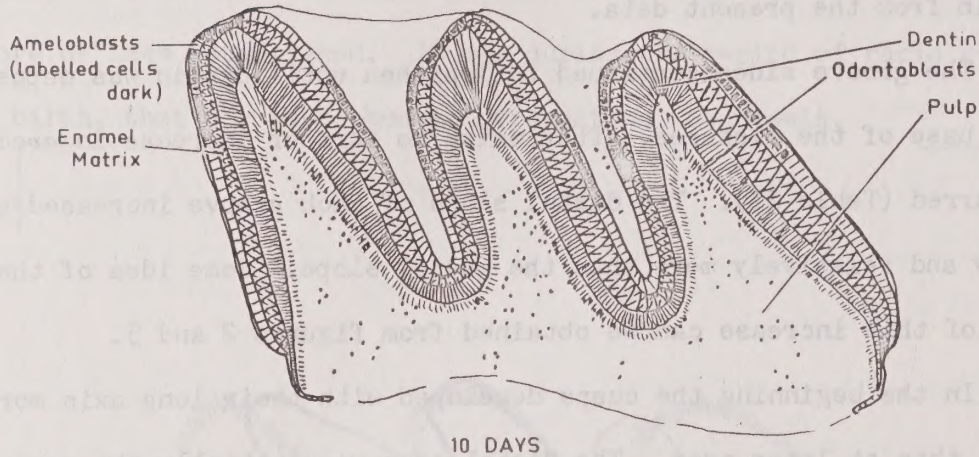
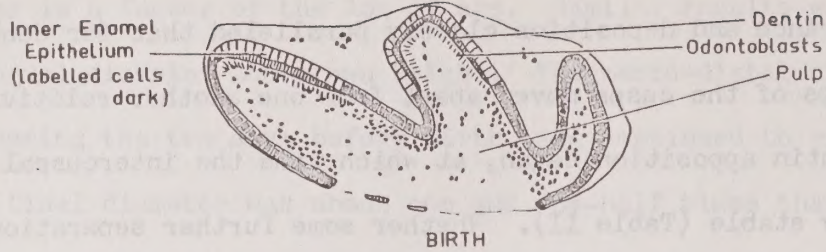


Figure 3. Sketches of autoradiographs of sagittal sections of developing rat first molars from animals injected with tritium-labelled thymidine, to show sites of D.N.A. synthesis. Both animals were injected at birth and one killed one hour later, the other after ten days.

Lack of label in cells of stellate reticulum after one hour, and its presence at five hours, together with the great activity in the adjacent stratum intermedium, was indicative of migration into the former layer by cells from the latter.

Cells in the inner enamel epithelium synthesized DNA until just about the time that dentin was deposited adjacent to their pulpal surface. When this happened no further division occurred in these cells, and those that had been labelled earlier, retained the label throughout anagenesis.

The line between those inner enamel epithelium cells that were labelled and those that were not, was quite sharp and was located close to the area where the basement membrane separating these cells from the pulp, became masked histochemically by the deposition of dentin matrix.

Odontoblasts differentiated from relatively undifferentiated cells of the pulp in the area of the pulpal aspect of the cervical loop, and once they had differentiated to the point of synthesizing dentin, no further divisions occurred in them. Vascular proliferation throughout the odontoblast layer was evident.

An eruption mitoses occurred in very large numbers in the area of the root bifurcation in the periodontal membrane, adjacent to both root and interradicular bone. At the same time, cells external to the now reduced ameloblasts of the enamel organ underwent many divisions to form a thick stratified epithelial layer, which subsequently met and fused with the base layer of the oral epithelium. No part in this proliferation was played by the reduced ameloblasts, which could be followed histologically along the inner side of the gingival crevice next to the enamel. These cells became more reduced in size and their nuclei more pycnotic as they neared the gingival crest, where they were lost.

Discussion.

Both aspects of this study have provided some new insight into the nature of the tooth and its diseases. The confirmation of the vitamin D effect on caries susceptibility is of significance in human populations, particularly in this country where evidently a deficiency of this vitamin in child nutrition is far from uncommon (McHenry, 1961). Knowledge of the nature of the interaction between the vitamin and fluoride may help explain the variation in susceptibility in areas where water supplies are fluoridated. More information

of this nature should accrue as the study of the relation of calcium and phosphorus intake on the pattern of susceptibility continues.

By following the development of the rat molar as it grows, we have learned more exactly of the period during which factors must act if they are to be effective in changing tooth shape, and perhaps susceptibility. In the rat upper first molar this critical period is during the period from shortly before birth to about three days after when groove depth and the basic shape has been established. This study did not include a measure of the contribution that enamel deposition makes to final tooth form, which was thought to be of lesser significance since the basic form of the crown is determined from the shape at the dentino-enamel junction.

Of particular significance was the finding of the intimate relation between cell divisions in the inner enamel epithelium (i.e. the growth in size of this cell layer) and the deposition of dentin on its pulpal surface. Since the grooves continue to deepen primarily by mitotic activity of inner enamel epithelium, their final depth is determined by the interaction of cell divisions in an epithelial layer on the one hand, and the rate of connective tissue cell differentiation and dentin synthesis on the other. How this affects human tooth form and possibly caries susceptibility has recently been discussed at some length (Paynter and Grainger, 1962) and will but briefly be reviewed here.

One primary morphological difference between the form of rat and human molars is that in the rat, the grooves, which run bucco-lingually across the tooth, are not bounded on each side by a marginal ridge. Thus they probably act more as spillways than do the grooves in human teeth. On the other hand, the grooves running across human molars are bounded on the mesial and distal sides of the crown by high marginal ridges, and on the buccal and lingual sides by intercuspal ridges. In both instances the occlusal edge of the ridges are some distance above the deepest point on the crown surface.

Thus as dentin is deposited in the tips of the cusps, and then spreads out to unite the cusps, the first union occurs along the marginal ridges and buccal and lingual intercuspal ridges. Once this dentin is laid down and to some degree mineralized, no further increase in the overall size of the crown at the occlusal surface can occur. At this time the centre of the crown, i.e. grooves and fossae, are still composed of soft tissue, and the cells of the inner enamel epithelium in this site are still dividing, but now within a fixed boundary. At this stage something interfered with dentinogenesis without also reducing mitotic rate in this epithelial layer, this layer would in effect become "too big" for its boundaries, and would either bulge up to wrinkle the surface, or, more likely dip down to form a deeper groove. Conversely, if dentin apposition were speeded up without concomitant increase in mitotic rate, theoretically the grooves would be shallower.

A similar process affects the size of the crown around the cervix, where size is determined by mitoses in the epithelial cells of the root sheath or cervical loop, and dentinogenesis around the periphery of the crown. Factors causing deep grooves should thus result in teeth larger at the cervix. Of interest in this respect is the observation that the human lower first molars that show most pit and fissure susceptibility also tend to be larger at the cervix. (Paynter and Grainger, 1961).

Some further support for this hypothesis has been obtained in rats by injecting animals at birth with cortisone each day for several days, and then with tritium-labelled thymidine, and subsequently examining their teeth. Cortisone at this age is quite toxic, and preliminary studies led to animal death within about two weeks. The experimental teeth were affected morphologically, which may have been at least partly due to toxicity. However this was probably not the only reason since counts of labelled nuclei in various cell groups in the

developing teeth disclosed than while labelling in the cortisone-treated animal in most cell layers (toxicity?), in the inner enamel epithelium of this animal there were more labelled nuclei per section than in the control. Examination of sections revealed that dentin had not progressed down the cusp sides in the experimental tooth as in the control.

This hypothesis is of sufficient interest and potential value that more exploration along these lines should be done. The amount of information that can be obtained is limited however, and the study should be extended to include primate teeth. This creates further difficulties because of the limited material available, and the time involved in animal growth etc. Some exploration has been made to determine the value of the new world monkeys in such a study, which, if available could cut the time significantly. A study of their breeding habits, possibility for raising under laboratory conditions, etc., is currently under way in Houston, Texas, and more information will be obtained from there as it becomes available.

Immediate proposals for the study, are to complete the dietary experimentation now under way, and to continue the investigation of the mechanism of action of these factors affecting tooth form and susceptibility, not only in the simpler forms like rats, but also in primates, which are more directly applicable to humans.

K. J. Paynter.

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APPENDIX M

Progress Report to
ASSOCIATE COMMITTEE ON DENTAL RESEARCH
NATIONAL RESEARCH COUNCIL OF CANADA

on

Project DA-92

for research entitled

POLYVINYL-ALCOHOL SPONGE AND THE

HEALING OF BONY DEFECTS

by

A. H. Sinclair-Hall, M.D., F.R.C.S.

Assistant Professor

Department of Anatomy

Faculties of Medicine and Dentistry

University of Manitoba

Winnipeg, Manitoba

December, 1961

Progress Report to
ASSOCIATE COMMITTEE ON DENTAL RESEARCH
NATIONAL RESEARCH COUNCIL OF CANADA

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Department of Anatomy

Faculty of Medicine and Dentistry

University of Manitoba

Winnipeg, Manitoba

December, 1961

1. INTRODUCTION AND AIMS

Bony defects were produced surgically in the jaws of dogs; these defects were filled with polyvinyl sponge or left unfilled as contralateral controls.

The healing of these defects was studied by comparison of histological sections and also by intra-oral radiographs and clinical observation.

These studies are designed to assess:

(A) Specifically -

1. The reactions, if any, of osseous and other connective tissues to polyvinyl sponge and the sponge's effect on bony callus formations.
2. The value or role of polyvinyl sponge as a framework for ingrowth of soft tissue and bone.
3. The adhesion and physical bonding of polyvinyl sponge to bone.

(B) Generally -

The value of polyvinyl sponge as an aid in the rate and type of healing of tooth sockets and other bony defects in the jaws of dogs.

2. METHOD

Bony defects have been produced surgically in the jaws of 12 dogs. In all dogs the polyvinyl sponge was implanted into defects on the left and the control defects on the right were left unfilled (See Figure 1, page 8). The maxillary bony defects are extraction sockets in alveolar bone and the sponge implant is subgingival. The mandibular circular defects are drilled in compact basal bone and the implant is subperiosteal.

(a) Operative Procedure* (Figs. 2-5).

After raising a mucoperiosteal flap a circular defect was drilled in the mandible on each side (see Fig. 2).

Polyvinyl sponge was cut to shape and implanted in the defect on the left and the flap closed (Figs. 3 and 4).

Upper 2 incisors were extracted. Sponge was cut to socket shape and implanted on left. The gum was closed with a figure of eight suture. The socket on the right was left unfilled and the gum closed as on the left (Fig. 5).

(b) Killing Animals

Dogs were killed at the times shown in Table I, which is a summary of the observations made in this study to date.

(c) Preparation of Specimens

After the tissue blocks were fixed, decalcified and embedded they were sectioned vertically through the defect area. On the left side, in those cases in which polyvinyl sponge was not found, serial sections have been prepared and are being examined.

3. OBSERVATIONS

(a) Clinical

One dog (#5) died postoperatively of an anaesthetic overdose.

Of the remaining 11 dogs, 3 are alive and healthy and 8 remained healthy until killed.

The gingival and mucosal wounds healed rapidly except for occasional small areas where they became necrotic and ulcerated. The wounds were

*For further detail see "Outline of Method", attached page 1 to my application of December 20, 1960.

TABLE I
M-5

Dog #	(Weeks)		Polyvinyl sponge implant	Control defect	Microscopic		Clinical	Radiographic
	Age of implant and of unimplanted control				Control defect	Polyvinyl sponge defect		
#1	41		*Not found					
#2	61, alive							
#3	59, alive							
#4	36		Found only in maxilla	Mature bony trabeculae	Connective tissue and immature osseous invasion.		Healthy	More regular and dense trabeculae on control side.
#5	Died							
#6	32		Found only in maxilla	Mature bony trabeculae	Active osteoblastic differ- entiation. Immature bone and more primitive connec- tive tissue.		Healthy	More regular and dense trabeculae on control side.
#7	29		Found only in maxilla	Mature bony trabeculae	Mainly connective tissue reaction.		Healthy	
#8	23		*Not found					
#9	20		Found only in mandible	Mature bony trabeculae and small abscess.	Osteoblastic activity. Connective tissue and immature osseous invasion.		Healthy	
#10	19, alive							
#11	10		Found only in mandible	Mature bony trabeculae	Connective tissue and immature osseous invasion. Many giant cells. Cellular cementum, periodontal liga- ment and sponge in contact.		Healthy	
#12	9		*Not found					

*at present - further serial sections are required.

remarkably free of inflammatory reaction and no sinus formation occurred. The weekly examinations, after the wounds ^{were} ~~was~~ healed, revealed no appreciable difference in shape, contour or consistency of defect and implanted regions.

Photograph of dog #6 taken before killing (Fig. 6) shows scars of mucosal wounds and contour of gums and alveolar areas. Photographs of all other dogs are similar.

Polyvinyl sponge was not found in dogs #1, #8 and #12. The wounds in these 3 dogs showed ulcerated areas which healed within 2 weeks. The sponge was not seen in any of these areas but could have been extruded through them when the dogs were unobserved. Dogs #2, #3, and #10 are healthy and it is hoped will show any long term changes that occur in the tissues or sponge.

The implanted sponge was found in 5 of the 8 dogs killed. In dogs #4, #6 and #7 it was only found in the maxillary defects. In dogs #9 and #11 only in mandibular defects.

(b) Microscopic

See Figures 7-18.

Dog #4 - Figures 7-12.

Dog #6 - Figure 13.

Dog #7 - Figures 14 and 15.

Dog #9 - Figure 16.

Dog #11 - Figures 17 and 18.

(c) Radiographic

See Figures 19 and 20.

Dog #4- Figure 19.

Dog #6- Figure 20.

4. SUMMARY OF PRESENT OBSERVATIONS AND CONCLUSIONS

Observations to date suggest that:

1. Polyvinyl sponge stimulates the formation of collagenous tissue and delays new bone formation when implanted in bony defects in the jaws of dogs.

2. The implant defects of all ages contain a very high proportion of fibrous connective tissue, whereas control defects of similar ages contain little fibrous tissue.

3. New bone is formed more rapidly in polyvinyl sponge implanted subperiosteally in mandibular bony defects than in sponge implanted subgingivally in maxillary socket defects. New bone penetrates the connective tissue within the sponge at a slow rate.

4. The morphological appearance of the contact areas between bone and sponge in some areas is similar to that of a carpenter's dovetail joint. Mechanical bonding or anchorage between sponge and bone seems to occur.

5. The sponge causes minimal foreign body reaction in the tissues. This reaction is judged by the round cell infiltration and not by the presence of giant cells.

6. Giant cells are most prevalent in the 10 week specimen.

7. Any deterioration or absorption of the sponge occurs slowly.

8. Mineralisation of the borders of the sponge, as indicated by the extent of their basophilic staining, increases as the implant ages.

9. Polyvinyl sponge is seen in contact with an area of periodontal ligament which is reattaching to newly formed cementum lining a defect in dentin.

10. There were no malignant changes.

5. PROPOSED FUTURE RESEARCH

Further investigations will be carried out as at present on older specimens of implants especially with regard to deterioration of the sponge and as to what later balance is reached between connective tissue and bone.

Studies on younger specimens will also be made to determine the earlier tissue reaction to polyvinyl sponge and its effect on bony callus formation.

Radiographs will be taken before future operations.



FIG. 2

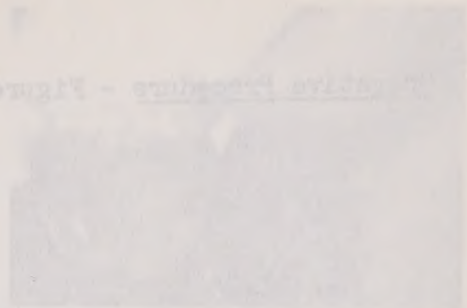


FIG. 3

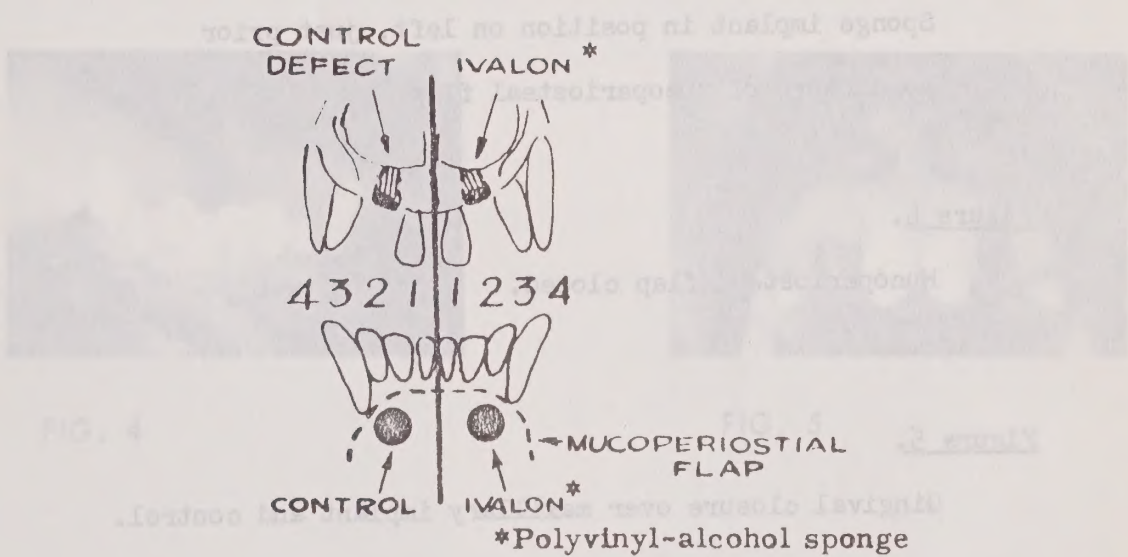


Figure 1.

The control and implant maxillary defects are in alveolar bone.

The control and implant mandibular defects are in compact basal bone.

Operative Procedure - Figures 2-5.

Figure 2.

Circular defects prepared in mandibular bone.

Figure 3.

Sponge implant in position on left, just prior
to closure of mucoperiosteal flap.

Figure 4.

Mucoperiosteal flap closed.

Figure 5.

Gingival closure over maxillary implant and control.

Figure 6.

Shows scars of mucosal wounds and contour of gums
and alveolar areas.



FIG. 2



FIG. 3



FIG. 4



FIG. 5



FIG. 6

Dog #4 - Figs. 7-12.

Figure 7.

Polyvinyl sponge maxillary implant defect after 36 weeks, showing alveolar socket area (H & E; x4.0).

The dark outlined masses of sponge are seen filling the sockets inside the cortical bone and extending through it at the crest. The light coloured areas are mature and immature connective tissue that have infiltrated the interstices of the sponge. The darker irregularly shaped areas are immature bone trabeculae growing from the margin of the defect. A few of these trabeculae have infiltrated to the center of the sponge.

Figure 8.

Control without implant defect after 36 weeks (H & E; x4.0).

Compared with Figure 7, the cortical bone is thicker, especially at the crest where the healing process is more advanced. Larger, mature but less numerous trabeculae are seen in the marrow spaces.

Figure 9.

Shows higher magnification of central area of implant defect (Fig. 7) (H & E; x25).

Figure 10.

Shows higher magnification of central area of control without implant defect (Fig. 8) (H & E; x25).

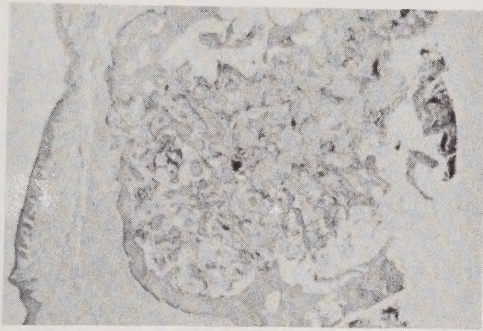


FIG. 7

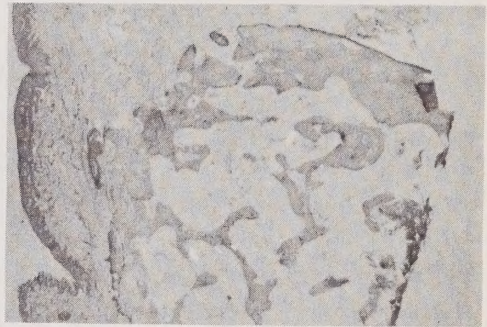


FIG. 8

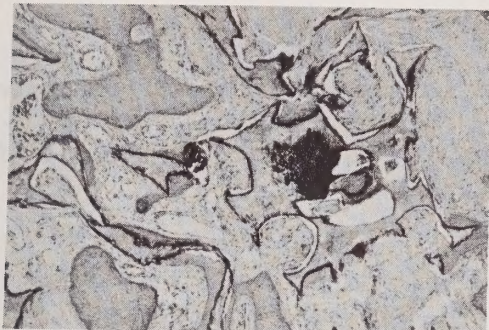


FIG. 9



FIG. 10

Figure 11.

The arrow indicates a boot shaped mass of sponge morticed into the endosteal surface of the cortical bone. Two circular areas of immature bone containing dark dots (osteocytes) are seen close by (H & E; x25).

Figure 12.

In the centre of the field an osteoclast in contact with the dark calcific border of the sponge, which appears fragmented in this area. Connective tissue is seen filling the continuous lacunae around the mass of sponge. The fine pores in the mass of the sponge are not infiltrated by connective tissue but with an eosinophilic homogenous substance or else are empty, (H & E; x205).

Dog #6Figure 13.

Sponge implant after 32 weeks (H & E; x25).

In the centre of the field a wing shaped mass of sponge can be seen extending completely through the cortical bone. The periosteal surface of the cortical bone is at the bottom of the section. Active osteoblastic differentiation is in progress. A more primitive type of connective tissue and more calcification are present than found in the 36 week implant defect.

Dog #7 - Figs. 14 and 15.Figure 14.

Polyvinyl sponge maxillary implant defect after 29 weeks (H & E; x25).

Mainly a connective tissue reaction is in progress. The connective tissue is growing into the lacunae of the sponge. Little bony reaction can be seen where the sponge is in contact with the bone. The calcific thick dark border of the sponge seen in the older implants is now much thinner.



FIG. 11

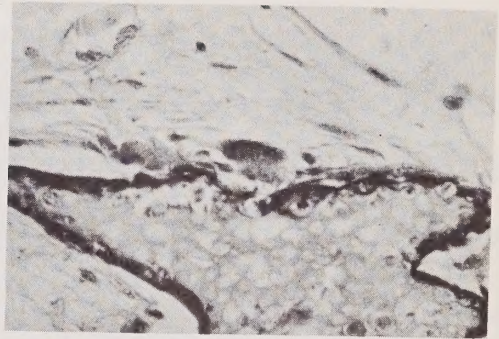


FIG. 12

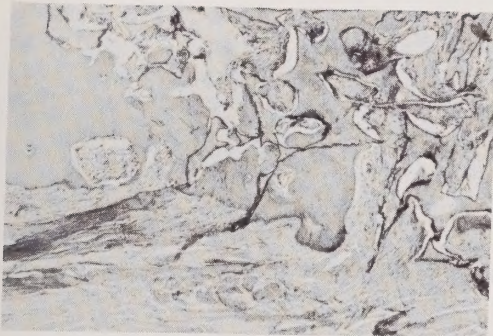


FIG. 13



FIG. 14

Figure 15.

Part of the same area (as Fig. 14) under higher magnification (H & E; x51).

Dog #9.Figure 16.

Polyvinyl sponge ^{mandibular}maxillary implant defect after 20 weeks (H & E; x25).

This implant is bedded subperiosteally in compact mandibular bone and although the implant is nine weeks younger than that seen in Figs. 14 and 15 it shows osteoblastic activity everywhere between the young bony trabeculae. Osteoblasts and osteoclasts are seen throughout.

Dog #11 - Figs. 17 and 18.Figure 17.

Polyvinyl sponge mandibular implant defect after 10 weeks (H & E; x4.0)

This defect passes completely through the cortical bone and cementum into the dentin of a root. The arrow points to a trabecula of newly formed bone opposite the margin of the baylike defect in the dentin. This same trabecula can be recognised in the next section. The parallel collagenous fibers of the periodontal ligament line the surface of the cellular cement where they are also in contact with the sponge. Fibrous tissue lines both sides of the bony defect and invades the sponge.

Figure 18.

Polyvinyl sponge mandibular implant defect after 10 weeks (H & E; x25).

Throughout the defect connective tissue invades the lacunae of the sponge. Many giant cells are present in little spaces on the margins of the sponge. The border of the sponge is thin and less calcific. The arrow indicates a corner of the baylike recess in the dentin repaired with cellular cementum. Other prints, not available at present, show cementocytes in the newly formed cementum extending along the margin of the defect. Along the outer border of the cementum cementoblasts lie next to the reattached periodontal membrane which in several areas has polyvinyl sponge in contact with it and possibly the sponge is stimulating the reattachment.

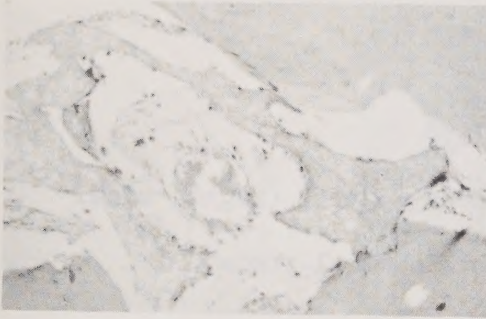


FIG. 15

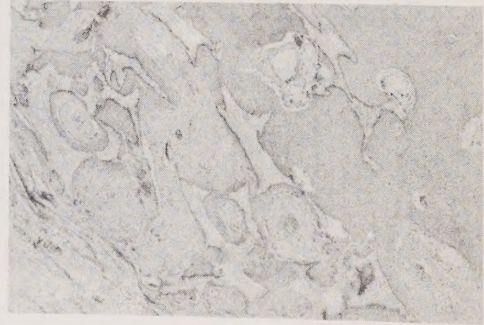


FIG. 16

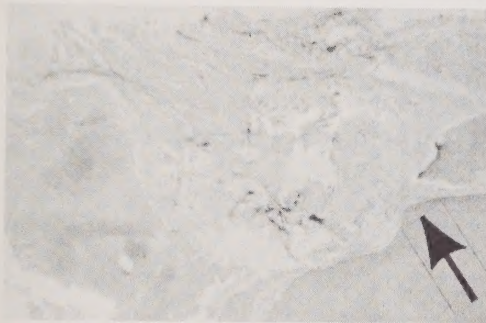


FIG. 17

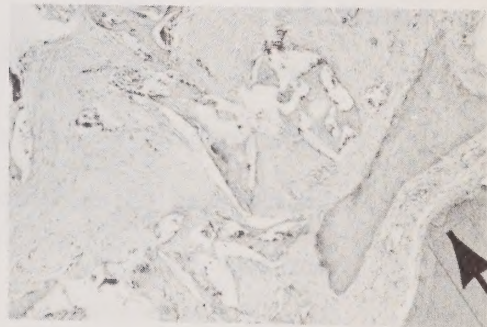


FIG. 18

Positive prints of intra-oral radiographs (x4).

Figure 19.

Dog #4, 36 weeks.

Some root resorption right central.

More regular and dense trabeculae on control (right) side.

Periodontal membrane thickened both central roots.

Figure 20.

Dog #6, 32 weeks.

More regular and dense trabeculae on control (right) side.

Thickening of periodontal space on apical third of left
central incisor.



FIG. 19



FIG. 20

APPENDIX N

PROGRESS REPORT ON PROJECT DA-93

John D. Spouge

Main Project

Work was commenced in May 1961 upon the project as outlined in the original N.R.C. application. The basic problem posed was to endeavour to ascertain why the histopathological picture of chronic marginal Periodontal disease seen in experimental animals (other than monkeys) differed from that found in humans. In animals such as rodents, resorption of bone and apical proliferation of epithelium does occur, but recession of the gingivae tends to keep pace with this bony resorption, and therefore the deep pocketing and round-cell response so characteristic of the human disease is absent.

One possible reason for this difference is the application of oblique stresses on the teeth resulting from the lateral movements permitted by the human temporal mandibular joint, especially in those dentitions with steep unworn cusps or occlusal irregularities. It was postulated that this may be the absent factor which results in the difference seen in the rodents and lower mammals, with their hinge-like temporal mandibular joints. Therefore the project sought to test this theory by applying varying degrees of artificial lateral stress to a dog's dentition, whilst exposing it at the same time to the irritation of our soft stagnation 'civilized' diet, and thus to assess the effect produced.

The following groups of animals were accordingly arranged, and the work as described was carried out:-

CONTROL ANIMALS

Group 1 - (2 dogs)

These were the basic control animals. They were fed upon a NORMAL HARD DIET. The animals were sacrificed at the end of 3 and 5 months respectively.

Group 2 - (2 dogs)

These animals were fed upon a SOFT NUTRITIONALLY ADEQUATE DIET, but their teeth were not subjected to any artificial trauma. The animals were sacrificed at the end of 3 and 5 months respectively.

EXPERIMENTAL ANIMALS

Group 3 - (2 dogs)

These animals were fed upon SOFT NUTRITIONALLY ADEQUATE DIET. In addition they were fitted with METAL CROWNS on their teeth, and these were designed only to make heavy contact during the late stages of the 'scissors' action of the masticatory excursions of the animals jaws.

Difficulty was initially experienced in obtaining adequate retention for the crowns on the short conical molar teeth, as the animals chewed extremely hard in an effort to remove the source of irritation. Therefore the longer canine teeth were used, and their shape provided a greater degree of retention. One metal crown was fitted to a canine tooth in each experimental animal.

Initially this appeared to produce the desired degree of lateral stress upon the opposing canine tooth, but after a period of two or three weeks it was considered that the animals were adopting a 'bite of convenience', and that they were avoiding placing stress upon the teeth involved. Therefore, whilst these two animals were persisted with, and sacrificed at the end of approximately 3 and 5 months respectively, it was decided to seek alternative methods to produce the lateral trauma which was desired. It was also decided to apply an additional artificial irritant to the surface mucosa, as an additive and alternative to the soft diet which was fed to the animals. Group 4 was thus utilized for the modified experiment.

Group 4- (2 dogs)

These animals were fed upon SOFT, NUTRITIONALLY ADEQUATE DIET. They were anesthetized at weekly intervals for a period of 6-8 weeks. During this operating session they each had a controlled degree of LATERAL TRAUMA applied to selected molar teeth by means of a surgical 'hand impactor' (a type of automatic mallet).

The object of this was to produce localized damage to the superficial portion of the periodontal membrane and associated alveolus. It was hoped that if the adjacent epithelium were also being irritated, then the localized inflammation resulting would prevent regeneration of the bone and periodontal membrane at the crest of the

alveolus; and that the apical epithelial hyperplasia allowed by this destruction of periodontal fibres, would produce the deep pocketing seen in human forms of chronic marginal periodontitis; and that the stagnation and infection within this pocket would result in the typical cellular infiltration.

To ensure this surface irritation, not only was the soft stagnation diet fed, but also known irritants (Croton Oil, and Oil of Turpentine) were applied locally to certain of the traumatized teeth, using adequate controls. The above animals were sacrificed after approximately 2 months.

Histological preparation of the experimental material is at present being undertaken. Due to the lengthy period of time required for decalcification, insufficient progress has been made for any conclusive opinion to be expressed at this stage.

It is hoped that if this alternative method of repeatedly applying controlled trauma to the animals' teeth (Group 4) shows any promise in producing a histological picture coinciding more closely with the human disease, then extended work will be continued on these lines during the Summer of 1962, and an effort made to assess the contribution of varying degrees of severity of the trauma.

Adequate funds still exist from the original Grant to enable continuation of this project, provided that the labour of one assistant is made available (see attached form N.R.C.603).

SUBSIDIARY WORK

As the above project entailed periods of activity limited to one or two days per week, and as the literature had already been thoroughly reviewed upon this topic, it was decided that two further simple projects could be undertaken with the resources and labour available.

Minor Project 'A'.

It was reported in the literature (B.D.J. Feb. 2, 1960 - H.G. Orlay - Endodontic splinting treatment in periodontal disease) that a successful clinical series had been carried out, involving the stabilization of individual teeth which had loosened by advanced periodontal disease. As an alternative to the more complicated multiple 3/4-Crown external splints, the writer had endeavoured to produce stability in the affected teeth by the use of stainless-steel posts, which were driven through the previously prepared root-canals into the apical bone, in an effort to surgically 'pin' the teeth (a technique which is the equivalent to that used in open operations to stabilize fractures in orthopedic surgery).

Since no histological investigation appeared to be either proposed, or to have been carried out by the author to check the effect of these manoeuvres on the apical tissues, it was thought that an equivalent animal study would provide a simple and useful project.

Therefore the incisor teeth of a number of dogs were prepared, and posts composed of varying materials were

inserted through the apices to penetrate the surrounding alveolar bone. The materials used consisted of 2 posts of stainless-steel, 2 posts of heat-cured acrylic, and 2 posts composed of dentine which had been 'turned' from extracted teeth, on a lathe. The workers on the project considered these latter of especial interest, as the possibility existed that Cementum may be deposited on this physiological tissue and a functional periodontal membrane could conceivably form around it (as occasionally happens on the Dentine-stump of successful apicoectomies).

The animals used in this project were sacrificed after approximately 10 weeks and the material is at present being decalcified in preparation for microscopic examination.

Minor Project 'B'.

It has long been considered possible that hypersensitivity may be one etiological factor responsible for certain forms of oral ulceration ('Canker sores', or 'Apthous ulcers'). Hypersensitivity tends to manifest itself as a 'diathesis', and a person with this predisposition frequently shows multiple manifestations of the upset (e.g. asthmatics have an increased tendency to become sensitized to drugs, antibiotics etc.).

It was therefore decided to check if any significant statistical relationship could be found between people who suffered from established forms of hypersensitivity reactions, and patients suffering from these recurrent oral ulcerations.

A questionnaire of the attached type was designed and carried out, utilizing an unselected group of 300 patients at the Manitoba Dental College and the Winnipeg General Hospital.

It was found that a statistically significant correlation did in fact exist between the two groups, and a paper is at present in process of being prepared with the object of demonstrating this fact, and also to clarify and publicizing to the Dental profession the modern theories governing hypersensitivity, and the appearance of lesions produced within the oral cavity.

It is hoped to do further investigations on this subject during the Summer of 1962, possibly involving both animal work and also the sensitivity tests on the oral tissues of affected patients.

To facilitate an exhaustive histological study of aphthous ulceration in particular and hypersensitivity diseases in general, it was considered that recourse to research through the files of a large Institution was desirable. Therefore the Registrar of the Armed Forces Institute of Pathology, Washington 25, D.C. was contacted and permission obtained provisionally to obtain facilities for study at this Institution for a period of 2 weeks during the Summer of 1962. In view of this, and the fact that no travel allowance had been utilized during 1961, permission is requested for a Travel and Subsistence Grant of \$375.00. (see attached form N.R.C.603)

REPORT ON GRANT N.R. - 85

"AN HISTOCHEMICAL INVESTIGATION OF THE GLYCOGEN CONTENT
IN THE DEVELOPING GINGIVA"

from

J. R. Trott

Faculty of Dentistry, University of Manitoba, Winnipeg

CONTENTS OF REPORT

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TO: THE NATIONAL RESEARCH COUNCIL OF CANADA

FROM: J.R. TROTT. FACULTY OF DENTISTRY, UNIVERSITY OF MANITOBA, WINNIPEG

REPORT: GRANT NR 85 "AN HISTOCHEMICAL INVESTIGATION OF THE GLYCOGEN

CONTENT OF THE DEVELOPING GIVGIVA".

The work which was started by the means of this grant in 1959-60 was completed in 1960-61, and a paper is being published in 1962 in the Journal of Histochemistry and Cytochemistry on "An Evaluation of Methods Commonly Used for the Fixation and Staining of Glycogen". A summary of this paper is attached. (Appendix A)

From this work the effect of various decalcification agents was tested on rat livers which had been adequately fixed as shown in the previous work. The results of this work are being published in the Journal of Histochemistry and Cytochemistry in an article entitled "The Prescence of Glycogen in the Rat Liver following "in vitro" processing in Decalcifying Agents". A summary of this paper is attached. (Appendix B)

When this work was applied to the developing gingivæ of rats, glycogen still could not be consistantly demonstrated. So a chemical analysis of the problem was carried out at the same time as the histochemical work was done. The results of this work are being published in the Journal of Histochemistry and Cytochemistry in an article entitled "A Chemical and Histochemical Investigation of Glycogen in Rat Livers and Palate following treatment with Various Fixatives and Ethylenediamine Tetracetic Acid." A summary of this paper is attached. (Appendix C)

Reprints of the three papers will be forwarded when they become available.

On the whole the work on this project has not progressed as favourably as the grantee had hoped. As will appear from the papers there are quite a number of problems to be solved first before one can go ahead with the original project of investigating the glycogen content of the developing gingiva.

It is therefore intended to suspend work along these lines temporarily, and to proceed with the work applied for in the accompanying application.

SUB-APPENDIX A

"AN EVALUATION OF METHODS COMMONLY USED FOR THE FIXATION AND STAINING
OF GLYCOGEN."

JOURNAL OF HISTOCHEMISTRY AND CYSTOCHEMISTRY

S U M M A R Y

Blocks of liver from eight, one-month-old Sprague Dawley rats were fixed in the following fixatives: - 10% neutral formalin, 10% formalin, formol saline, alcohol formalin, acetic alcohol formalin, Bouin's, Carnoy's and Rossman's for the following times: - 24 hours, 48 hours, seven days, two weeks, one month and three months. Sections were cut from those specimens and stained by the P.A.S., and the Best carmine method for glycogen. (Table I)

It is concluded from these experiments that if the tissues are fixed in acetic alcohol formalin, then consistently good results will be obtained with the P.A.S. stain, to demonstrate glycogen in livers, which have been stored in this fixative up to a period of three months. Nearly as good and consistent results can also be obtained with the Best carmine stain, if the tissues are fixed in Bouin's over a similar period of time. Aqueous based fixatives cannot be relied upon after 24 hours to 48 hours, to demonstrate the maximum amount of glycogen in tissues.

SUB-APPENDIX A-2

It was found that acetic alcohol formalin fixed tissues gave the most marked polarisation whilst sections taken from specimens fixed in aqueous based fixatives show considerably less polarization, and in some cases none at all.

Alcohol based fixatives give a coarser and larger granule of glycogen which stains with more vividness, and is easier to recognize following the P.A.S. reaction than the Best carmine.

An unusual distribution of glycogen was observed with the two stains that were used. With the P.A.S. reaction, some sections showed a very marked heavy peripheral accumulation of glycogen within the cells, whilst with the Best carmine method some sections showed a glycogen free peripheral zone. They were consistently reproduced but no suitable explanation can be offered for these observations.

This paper has now been published in the Journal of Histochemistry and Cytochemistry 9:703 (1961). Reprints are not yet available, but will be forwarded as soon as possible.

SUB-APPENDIX A-3

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T A B L E I

AMOUNT OF GLYCOGEN * P.A.S. STAIN AND BEST CARMINE STAIN

FIXATION TIME	24 hrs.		48 hrs.		7 days		2 weeks		1 month		3 months	
	P.A.S.	BEST	P.A.S.	BEST	P.A.S.	BEST	P.A.S.	BEST	P. A. S.	BEST P.A.S.	P.A.S.	BEST
STAIN												
Neutral 10% Formalin	+++	+	++	++	+	+	+	+	++	+	+	+
10% Formalin	+++	+++	++	++	++	+	+	++	+	+	+	O
Formol Saline	+++	+++	++	++	+	+	+	++	+	+	+	O
Alcohol Formalin	+++	+++	++	++	+++	++	+++	++	++	++	+++	++
Acetic Alcohol Formalin	+++	+++	++	++	+++	++	+++	++	+++	++	+++	++
Eosin's	+++	++	++	++	+++	++	++	++	++	++	++	++
Carnoy's	+++	++	++	++	+++	+	+++	++	++	+++	++	++
Rossman's	+	++	++	++	+++	++	++	++	++	++	++	++

SUB-APPENDIX B

THE PRESENCE OF GLYCOGEN IN THE RAT LIVER FOLLOWING "IN VITRO"PROCESSING IN DECALCIFICATION AGENTSJOURNAL OF HISTOCHEMISTRY AND CYSTOCHEMISTRYS U M M A R Y

The problem is to find a method for decalcifying jaws, which have been fixed to retain maximum amounts of glycogen in the gingiva. A liver was taken from a 1 month old Sprague-Dawley strain male rat, divided into 4 pieces and placed immediately in acetic-acid alcohol formalin and fixed for 24 hrs. The pH of the fixative was 4.0. After fixation one piece of tissue was processed immediately. Sections were stained by the P.A.S. reaction. Of the remaining three pieces of liver, one was placed into 5% trichloroacetic acid at a pH of 0.6, another in formic acid and sodium citrate at a pH of 2.5, another in E.D.T.A. buffered with NaOH to a pH of 7.5, for 7 days.(Table I) Later, another 6 week old Sprague-Dawley strain male rat was killed and the liver carefully sliced into pieces approximately 3mm. thick before being fixed in acetic-acid-alcohol formalin for 6 hrs., 24 hrs., 48 hrs., and 7 days. The specimens were divided into three groups. The first group were used as controls, and following fixation were processed as previously and the sections stained by the P.A.S. reaction.

SUB-APPENDIX B-2

Another group were placed in E.D.T.A. at a pH of 7.5 for 7 days.

The last group were placed in formic acid and sodium citrate, as in the previous experiment for 7 days. (Table II) It was shown that 5% Trichloroacetic acid, Formic acid and sodium citrate remove glycogen from specimens of liver following adequate fixation, and that E.D.T.A. is the only agent which retains adequate amounts of glycogen to be demonstrated histochemically. The length of fixation time did not appear to make any difference to the amount of glycogen retained in the liver.

This work was done on liver because the amount of glycogen in the gingiva was found to be small to act as an adequate control.

This paper has now been published in the Journal of Histochemistry and Cytochemistry 9:699-702.

Author	Year	Fixative	Glycogen
Scheer, J. H.	1957	Formic acid	+++
Scheer, J. H.	1957	Formic acid	+++
Scheer, J. H.	1957	Formic acid	+++
Scheer, J. H.	1957	Formic acid	+++
Scheer, J. H.	1957	Formic acid	+++
Scheer, J. H.	1957	Formic acid	+++
Scheer, J. H.	1957	Formic acid	+++
Scheer, J. H.	1957	Formic acid	+++
Scheer, J. H.	1957	Formic acid	+++
Scheer, J. H.	1957	Formic acid	+++

(1957) "An Investigation into the Glycogen Content of the Gingiva"

(1961) "An Evaluation of Methods Commonly used for the Fixation & Staining of Glycogen"

SUB-APPENDIX B-3

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SUB-APPENDIX B-4

TABLE I

Decalcification Agent	pH	Amount of Glycogen Present
5% Trichloroacetic Acid	0.6	0
Formic Acid & Sodium Citrate (Morse, 1945)	2.5	0
E.D.T.A.	7.5	+++
Control		+++

TABLE II

Fixation	Amount of Glycogen Present		
Acetic Alcohol Formalin	Control	E.D.T.A.	Formic Acid
6 hours	+++	+++	0
24 hours	+++	++	0
48 hours	+++	+++	0
7 days	+++	+++	0

SUB-APPENDIX C

A CLINICAL AND HISTOCHEMICAL INVESTIGATION OF GLYCOGEN IN RAT LIVER AND
PALATE FOLLOWING TREATMENT WITH VARIOUS FIXATIVES AND E.D.T.A.

JOURNAL OF HISTOCHEMISTRY AND CYTOCHEMISTRY

S U M M A R Y

From previous work it seemed possible that if small specimens of rat jaw were fixed in acetic alcohol formalin, decalcified in E.D.T.A. and stained by the P.A.S. reaction, glycogen would be observed in the developing oral and gingival epithelium. This did not however occur so both a chemical and histochemical analyses were carried out.

Total glycogen content of liver prior to fixation; after fixation in acetic-acid alcohol formalin; and 1% Periodic acid 10% formalin; and after seven days in E.D.T.A. were estimated by the anthrone method. Sections were made of the tissues and stained for glycogen. (Table I)

The loss of glycogen after fixation in acetic-acid-alcohol formalin as compared to immediate determination was found by chemical analysis to be from 5.2% to 13.8%. No appreciable difference could be seen histochemically.

Chemical analysis of the glycogen content of liver fixed in acetic-acid-alcohol-formalin and then treated in ethylenediamine tetracetic acid showed a total drop of 20.7% to 33.7%. Glycogen could still be adequately shown histochemically.

SUB-APPENDIX C-2

Sections taken from liver fixed in 1% periodic acid in 19% formalin show greater amounts of glycogen, than those taken from acetic-acid-alcohol-formalin fixed tissue.

After fixation in 1% periodic acid in 10% formalin and following treatment with ethylenediamine tetraacetic acid, very little glycogen could be demonstrated histochemically.

In the palate, no glycogen could be demonstrated after fixation in acetic-acid-alcohol-formalin, whereas, quite heavy amounts could be seen in the prickle cell layers of the epithelium following fixation in 1% periodic acid in 10% formalin.

Neither acetic-acid-alcohol-formalin nor 1% periodic acid in 10% formalin are satisfactory fixatives when the tissue is to be further decalcified in ethylenediamine tetraacetic acid, and if there are only small amounts of glycogen present.

No adequate method of retaining glycogen in small amounts in tissue requiring decalcification can be suggested.

SUB-APPENDIX C-3

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TABLE I

GROUP	NUMBER OF RAT	WEIGHT OF LIVER (grams)	SOLUTIONS	GRAM PERCENTAGE OF GLYCOGEN	HISTOCHEMICAL RESULTS
A	1	10.07	30% K O H	5.81%	
	2 (a)	1.89	30% K O H	7.06%	
	3	7.16	A A F - 6 hrs	5.01%	+++
B	2 (b)	1.96	A A F - 6 hrs	6.70%	+++
	4	7.53	A A F - 6 hrs EDTA - 7 days	3.85%	++
	2 (c)	1.41	A A F - 6 hrs EDTA - 7 days	5.60%	++
C	5	10.38	1% Periodic Acid- 6 hrs in 10% Formalin	2.98%	+++
	2 (d)	2.57	1% Periodic Acid-6 hrs in 10% Formalin	0.21%	+++
	6	8.38	1% Periodic Acid in 10% Formalin - 6 hrs EDTA - 7 days	1.75%	+
	2 (e)	1.05	1% Periodic Acid in 10% Formalin - 6 hrs EDTA * 7 days	0.0006%	+

APPENDIX P

FUNDAMENTALS OF AMALGAM METALLURGY AND THE DEVELOPMENT OF DENTAL AMALGAMS

NRC Grant DA-95

Progress Report 1961-62

by

D. B. K. Innes and W. V. Youdelis

ABSTRACT

This report outlines apparatus and procedures developed during a preliminary investigation of dental amalgams. Attempts have been made to improve the properties of amalgams by dispersion hardening and solid solution hardening. The results of specific alloy systems which have been studied are discussed.

PREFACE

This report is compiled from studies of the effects of copper and cadmium in dental amalgams. Work was carried out in the laboratory of the Department of Mining and Metallurgy, University of Alberta in Edmonton during the period May 1, 1961 to September 15, 1961. The work was supervised by Assistant Professor Dr. W.V. Youdelis of the Department of Mining and Metallurgy and was supported under the auspices of the National Research Council of Canada, Grant NRC DA-95.

INTRODUCTION

Several common metals dissolve in mercury to form alloys which are called amalgams. Amalgams which exhibit good compressive strengths, high hardness after aging, and good corrosion resistance to mouth fluids are suitable as dental restorative materials. For this purpose they must also have a fairly high initial hardness and a fast hardening rate, and must be easily prepared by the dentist. At present, dental amalgams are prepared by mixing equal weights of mercury and a powder alloy with a composition of about 65-75% Ag, 25-26.8% Sn, 0-2% Cu, and traces of zinc. Some of the properties of amalgams are very similar to those of concrete. Amalgams require aging to develop full strength; also, they are strong only in compression and fail with the characteristic cone failure of concrete. Considering mechanical properties, a properly prepared amalgam should have an ultimate compressive strength of about 40,000 pounds per square inch (p. s. i.) and a Diamond Pyramid Hardness (D.P.H.) of about 100.

This report deals with two methods which were used to try to improve the compressive strength and hardness of amalgams.

The first method involves the principle of solid solution hardening of alloys. Hardening in amalgams is due principally to the formation of the mercury-silver compound Ag_2Hg_3 . In metallurgical parlance this is a type of electron compound (the electron to atom ratio is presumably 8:5) having some degree of covalent character to its

bonding. It is known that the smaller the atomic number of the component atoms the stronger is the covalent bond. To increase the strength of the amalgam, therefore, an attempt should be made to replace some of the mercury in the hardening compound by an element having the same number of valence electrons but a lower atomic number. Cadmium is one such element. Zinc is also a possibility but with zinc there is the difficulty of an unfavorable atomic size factor. Cadmium has an atomic size that is much closer to mercury and should therefore tend to replace mercury better.

The second method of hardening of amalgams involves the principle of dispersion hardening. The basis of this method is to disperse small particles of hard phases throughout the amalgam which will bond to the matrix and increase the overall compressive strength and hardness of the amalgam. The theory is that the particles impede the movement of dislocations in the matrix and thereby increase the resistance to flow of the material when under stress. The silver-copper phase was chosen as the dispersent phase because of its high hardness and ability to form a diffusion ^{bond} through partial amalgamation. The matrix phase used was the standard amalgam of Ag_3Sn plus Hg in a 1:1 ratio.

APPARATUS AND PROCEDURES

1. Alloy Casting: Two main problems were encountered in casting: first, attaining a high enough temperature to melt the silver alloy, and second, preventing the melt from oxidizing.

The melting furnace utilized a hollow, one inch outside diameter vitreous silica heating tube, around which was wound a resistance wire heating element. The element wire was 1.15 ohms per foot Kanthal ribbon. A thirteen foot length was sufficient to wind a heating tube six inches in length. Total resistance was about 15 ohms, allowing passage of eight amperes at 120 volts. Because the element wire becomes embrittled by the quartz at high temperatures, it is necessary to coat the tube before winding the element. Sairset refractory cement was used for the coating material. A second coating was applied over the wound element to seal it in. The tube cracked after several heating cycles, but this does not impair the operation of the furnace. The tendency of the tube to crack could be decreased by increasing the time of heating to and cooling from the desired temperature.

Preparation of the alloy was done in Alundum-coated, graphite crucibles. Copper molds were satisfactory for silver-tin alloys, but silver copper alloys require a lined mold to prevent the melt from alloying with mold. Molybdenum foil or a thin coating of

Alundum cement were found to be satisfactory for the purpose.

Oxidation was prevented by introducing a stream of argon gas into the crucible during melting. Stirring and degassing were accomplished by bubbling argon gas through the melt.

2. Powdering and Sizing: In considering properties of amalgams the particle size appears to be an important factor. There is an optimum size which gives the best mechanical properties to the amalgam.

For brittle materials such as silver tin alloys, powdering is easily accomplished by turning the ingot on a lathe. A slow rotation speed and use of a sharp pointed cutting tool give a suitable powder.

For more ductile materials such as the silver copper alloys and low cadmium content silver-cadmium alloys, a clean sharp file seems to be the best method for powdering the material, but the particles have a sliver shape which increases the difficulty of size screening.

The surface scales should be removed from the ingot before powder is prepared.

Only small volumes of the powder should be sized at a time if a complete sizing is to be effected.

3. Amalgamation and Specimen Preparation: The amalgamation procedure adopted in this investigation consisted of triturating for a predetermined length of time, squeezing the material in linen cloth (for one minute), packing the material in molds and finally compressing the material in the mold at a predetermined pressure for one minute.

The hardness test specimens were prepared in a 1/4" diameter hole drilled 1/4" deep in a Bakelite plug. Compressive strength test specimens were of two sizes, one-quarter and one-eighth inch diameters.

Packing pressures in early experiments were applied by a metallurgical press (24,000 psi) but were not accurate. Later specimens (#36 on) were compressed in a Hounsfield Testing Machine (10,000 psi) which provided much higher accuracy. To obtain a pressure of 10,000 psi, a force of 491 lbs. is required on quarter-inch molds and a force of 123 lbs. on eighth-inch molds.

Generally, about 1.5 grams total weight of mercury and alloy powder was required to prepare an eighth-inch diameter compression test specimen and a minimum of five grams was necessary for the quarter-inch specimens.

4. Testing: (a) Hardness Specimens - After preparation, the specimen was left to set for several minutes after which it was polished. Final polishing was done on napless cloth using ethanol as a lubricant. Water severely attacks the amalgam, especially in the early stages of setting. To etch a specimen, it was only necessary to immerse it in warm water for a few seconds. The surface was then flushed with ethanol to prevent subsequent attack by moisture.

Hardness testing was commenced as soon as possible after preparation of the amalgam. The Tukon hardness tester with a Diamond Pyramid indenter was used. Tests were made at close intervals (ten minutes) for the first hour, at fifteen or thirty-minute intervals for the second hour and every hour thereafter when possible. Aging time is considered to commence when the specimen is removed from the Hounsfield machine.

(b) Compression Test Specimens - These specimens were allowed to set in the molds for ten minutes after the molds were removed from the Hounsfield Machine. At this point, the ingot was ejected from the mold and placed under the dilatometer to measure the longitudinal expansion of the amalgam. Readings were taken at close intervals at first, in order that any contraction could be noted.

When the maximum hardness of the amalgam had been attained, the compression test samples were tested in the Hounsfield machine. A most important factor in preparing the test sample is to

ensure that the ends of the specimen are flat and normal to the longitudinal axis of the sample. It is equally important that the ratio of the diameter to the length of the specimen be as close to 0.5 as possible.

Two loading rates were used in compression tests. These were, in terms of extension, 1/16 inches per minute and 1/8 inches per minute. These are termed slow and fast loading respectively in this report and, unless otherwise noted, all loading rates used were fast (1/8 inches per minute).

RESULTS AND DISCUSSION

1. Solid Solution Hardened Amalgams: For the first amalgams studied (#1 to #35 inclusive) the condensation pressures are not known with high accuracy, but are of the order of 24,000 psi. Where the hardness test was considered to sufficiently evaluate a material, a compression test was not made. Only those results from amalgams which were condensed in the Hounsfield machine can be considered as reproducible and consistent. All others may be viewed as indicators and for which more precise determinations are necessary.

In this report, maximum hardness refers to the maximum value obtained, irrespective of the aging time. In some cases there are wide variations in hardness of an amalgam and this is attributed to the inhomogeneity of the microstructure.

The standard to which all experimental results have been compared is an amalgam of the 75Ag-25Sn alloy powder. This alloy composition gives the gamma phase constituent in the silver-tin system. The alloy has a hardness of 137 DPH and readily powders.

Table I gives the hardness values and compressive strengths of standard amalgams prepared under varying conditions of time and pressure. Clearly illustrated (in S. 1 and S. 2) is the relationship between the ultimate compressive strength and the condensation pressure used in preparing the amalgam. The relationship between

TABLE I

Standard Amalgams

No.	Composition and Remarks	Hardness (DPH)	UCS (p. s. i.)
8	Ag ₃ Sn, 160% Hg, 250 mesh Triturated 2 minutes	106.7	48,800
12	Same as #8 but using 325 mesh powder	93.5	49,800
20	Ag ₃ Sn, 100% Hg, 250 mesh Triturated 4 minutes	87.9	--
S. 1	Ag ₃ Sn, 100% Hg, Trit. 2 min. Condensed at 40,000 psi	-	54,800
S. 2	Same as Standard #1 (S. 1) but condensed at 10,000 psi	-	44,600 slow loading
S. 4	Same as S. 2 but loaded fast	-	49,000
S. 3	Same as S. 2	91.3	--

compressive strength and loading rate is also apparent (in S. 2 and S. 4.

Characteristics of the stress-strain diagrams of compression indicate that very little flow occurs in fully aged amalgams of this type. There

also appears to be very little difference in hardness and compressive strength whether the amalgam is triturated for two, four or seven

minutes. Initial testing showed that a 1:1.6 powder to mercury ratio provided too much excess mercury; subsequent tests were made using

a 1:1 ratio. This still left a slight excess of mercury and made the material much more workable.

TABLE II

Pure Powder Amalgams

No.	Composition and Remarks	Hardness (DPH)	UCS (p. s. i.)
1	70 Hg - 30 Ag (wt. %)	85.8	30,000
2	65 Hg - 35 Ag	78.2	34,000
3	80 Hg - 20 Ag	75.2	--
4	50 Hg - 50 Sn	51.2	Flowed
5	70 Hg - 30 Sn	--	3,000
6	75 Ag - 25 Sn, 160% Hg	66.8	31,200
11	(65 Hg-35 Ag), 105% Ag, 160% Hg	54.8	33,300
13	(65 Hg-35 Ag), plus Ag, Hg	79.0	34,300
28	70 Ag - 30 Cd, 100% Hg	65.5	--

Note: Condensation pressures 24,000 psi

Particle size: -100 to +250 mesh material

The first new investigations made were to determine the usefulness of pure elemental powders in amalgams. Results of this test series are tabulated in Table II. Amalgams of pure silver powder, pure tin powder and mixtures of pure silver and tin powders, were prepared with compositions in the gamma phase region. Hardness values were in some cases fairly high but compressive strengths were low. In the case of pure tin amalgams, the material flowed so readily

that an ultimated compressive strength could not be determined. Tests #11 and #13 were made using a powdered 65 Hg- 35 Ag amalgam which was reamalgamated by adding more mercury and more silver powder. Amalgam #28 is not a true indication of the Ag-Cd system. The cadmium powder used was in a very coarse, slivery form and did not fully amalgamate. This test should be repeated using a finer cadmium powder.

TABLE III

Standard Powder and Pure Powder Amalgams

No.	Composition and Remarks	Hardness (DPH)	UCS (p. s. i.)
7	50% standard, 50% pure powders of 75Ag-25Sn comp. 160% Hg, trituated 7 minutes	73.9	41,500
9	Same as #7, but mixed in opposite order. Trituated 7 minutes	84.7	41,300
14	Standard plus 2.6% Cd powder 160% Hg	97.8	52,500
15	Same as #14, but Cd amalgamated first	96.3	--
16	Same as #15, but 5.2% Cd	88.8	--
17	Same as #16, but Hg added to mixture of both powders	84.0	--
18	Repeat of #14, trituated 7 mins.	86.6	--
19	Repeat of #14, trituated 5 mins.	76.9	--

Note: Condensation pressure 24,000 psi. Particle size: -100 to +250
mesh material

The second series of new tests (Table III) employed mixtures of pure elemental powders with the standard 75 Ag-25 Sn cast alloy powder. Additions of tin and silver powders showed no decided advantages over standard amalgams but small quantities of cadmium powder gave a marked increase in compressive strength. Here again, difficulty was encountered in amalgamating the coarse cadmium powder. It is difficult to prepare pure cadmium powder as cadmium metal is very soft.

In continuing the study of replacing mercury with cadmium, a series of amalgams (Table IV) was prepared using AgSnCd alloy powders. Alloys were cast using a 75 Ag-25Sn composition with 5% and 10% cadmium added. Difficulty was experienced in melting cadmium because of its very high vapor pressure and low boiling point. Cadmium vapors are toxic, necessitating extreme caution when working with the liquid metal. Alloys #4 and #5 (containing 5% Cd) had a hardness of about 153 D.P.H. and were easily powdered. The 10% Cd alloy had a hardness of only 130 D.P.H. but still powdered easily. While amalgams #21, #22 and #25 showed fairly high final hardnesses, they are unsuitable because their hardening rates are very slow. However, amalgam #23 showed a high initial and a fast hardening rate. Compressive strength for one test specimen was 15% higher than that of a standard amalgam. No apparent advantage was gained by adding pure cadmium powder to the alloy powder as was done in amalgam #24.

TABLE IV

AgSnCd Alloy Amalgams

No.	Composition and Remarks	Hardness (DPH)	UCS (p. s. i.)
21	AgSnCd (Alloy #4), 100% Hg Triturated 4 minutes	85.1	--
22	AgSnCd (Alloy #4), 150% Hg Triturated 7 minutes	85.1	--
23	AgSnCd (Alloy #5), 100% Hg Triturated 4 minutes	101.8	50,400
24	AgSnCd (Alloy #5), 100% Hg plus Cd powder. Trit. 4 min.	37.2	--
25	AgSnCd (Alloy #6), 100% Hg Triturated 4 minutes	87.2	--
26	AgSnCd (Alloy #6), 200% Hg Triturated 4 minutes	39.1	--

Note: Condensation pressure 24,000 p. s. i.

Attempts were also made to amalgamate 70% Ag - 30% Cd alloy powders, but the hardness of this alloy was so low (53 D.P.H.) that it was impossible to prepare a suitable powder.

2. Dispersion Hardened Amalgams: In preparing to investigate the silver-copper amalgam system, studies were made of 92% Ag - 8% Cu (sterling silver) age-hardenable alloys. Powders of this alloy in the maximum hardness condition were amalgamated but proved unsuccessful because of a failure of the particles to amalgamate

fully. Following this, age-hardening studies were made of 71.9% Ag - 28.1% Cu eutectic alloys. The solubility limits of silver in copper and copper in silver (solid solutions) makes it possible to harden this alloy by forming precipitates in both the silver rich and copper rich regions. However, after solution heat treatment and aging, the resulting alloy was not quite so hard as the chill cast alloy so that the chill cast alloy was chosen for amalgamating. An attempt was made to amalgamate the silver-copper eutectic alloy powder itself without adding any standard Ag_3Sn powder. It was found that although hardness values were high and increased rapidly with aging time, the compressive strength was nil. This suggested that the amalgamation was only superficial, leaving the cementing phase between the particles too rich in mercury with very little strength.

In view of the fact that the AgCu particles could not cement together properly in an amalgam, a series of amalgams was prepared using the AgCu eutectic particles as a dispersion hardening constituent in a predominantly 75 Ag-25Sn standard alloy powder cementing matrix. Table V indicates the effects of the AgCu particles on the properties of the amalgam. Illustrated are: the effects of increasing the amount of dispersion powder, changes in particle size and effect of decreasing the mercury content of the amalgams. The general conclusion to be drawn from these results is that an amalgam containing 20% AgCu hardening particles of 200 mesh size in a standard alloy powder cementing matrix

TABLE V

Dispersion Hardened Amalgams

No.	Composition and Remarks	Hardness (DPH)	UCS (p. s. i.)
42	Standard powder plus 10% AgCu, 100% Hg, Alloy 11-as cast, 200 mesh powder, triturated 2 mins.	101.0	55,800
43	... 20% AgCu, 200 mesh	115.0	54,800
45	... 30% AgCu, 200 mesh	104.0	55,500
42b	... 10% AgCu, -325 mesh	83.0	40,250
43b	... 20% AgCu, -325 mesh	98.8	38,500
44	... 30% AgCu, -325 mesh	96.7	46,100
46	... 10% AgCu, 250 mesh	99.5	49,300
47	... 20% AgCu, 250 mesh	88.8	55,000
48	... 30% AgCu, 250 mesh	101.0	55,300
49	10% AgCu but only 90% Hg, 250 mesh	106.4	43,200
50	20% AgCu but only 80% Hg, ...	96.0	--
51	30% AgCu but only 70% Hg, ...	95.3	26,900

Note: Condensation pressure 10,000 p. s. i.

is the most suitable from the standpoint of hardness, hardening rate and compressive strength. The effect of particle size is not clear but smaller particles do appear to decrease the hardening rate of the

amalgam. Small particles (-325 mesh) also require more mercury to amalgamate them due to the increased surface area. Decreasing the mercury content (Amalgams #49-#52) by adding only as much mercury as there is standard alloy powder present as the cementing material, gives a marked decrease in compressive strength and hardening rate.

The results from this survey of copper and cadmium amalgams leave many questions to be answered before the usefulness of the materials can be decided conclusively.

Some cadmium amalgams show reasonable hardness (an increase in compressive strength of approximately 15% occurred in #14 amalgam) and compression strength while others, which differ only in mixing procedures, give poor results. Studies should be made using silver-cadmium alloys in the 41% to 45% Cd composition range. These alloys are very brittle and should give a good powdered alloy. The only difficulty foreseeable would be in amalgamating this material. This composition may also be useful as a dispersion hardening constituent.

With copper amalgams, the main problem is to get enough of the material amalgamated without using excessive amounts of mercury. Small amounts of these powders will readily amalgamate in a standard alloy powder cementing matrix. The dispersion hardening theory was well demonstrated in amalgam #43 and this composition should have good possibilities as a dental restorative material. The increase in compressive strength above that of the standard was a very

Project: DA-94

THE NATURE OF THE CARIOSTATIC ACTION OF DIETARY PHOSPHATES

by Jules Tuba, C.R. Castaldi, L.J. Heppler* and Morris Seneshen*

The work in progress at Alberta for the past two years is designed to determine whether the cariostatic effect of increased levels of dietary phosphate is due to "local" or "systemic" effects or both. Evidence in the literature seemed to favour the first.

Our first experiments were concerned with the effect of metaphosphoric acid in the diet on caries incidence in weanling hamsters (1). It was proposed a year ago to use metaphosphoric acid or a salt containing P^{32} to trace local or systemic effects. However, Atomic Energy of Canada, Chalk River, informed us that radioactive metaphosphate would be too unstable to use.

Experiment I

Consequently, we decided to use disodium hydrogen phosphate which had been used successfully by Nizel, Harris and Parker (2) in increasing caries resistance in the growing rat. Our investigation was with the weanling hamster and we studied the effect; not only of dietary supplements of Na_2HPO_4 , but of completely coating the supplementary dietary particles of phosphate to permit the salt to remain undissolved in the mouth for a maximum time yet not remain undissolved in the gastrointestinal tract.

* Dental Summer Students

Search for Coating Material for Na_2HPO_4

Fifteen different substances were examined as possible coating materials for the phosphate particles. These included: gelatin, ethyl cellulose in ethanol, arsenic-free shellac, white paraffin, collodion, gum acacia, casein plus ethanol, stearyl alcohol ($\text{C}_{18}\text{H}_{37}\text{OH}$) with cetyl alcohol ($\text{C}_{16}\text{H}_{33}\text{OH}$). A coating of the two last compounds, stearyl and cetyl alcohols proved to be the most valuable.

Coating of Na_2HPO_4

Na_2HPO_4 was screened to remove very fine or coarse particles, 400 gm. of intermediate size phosphate particles were moistened and stirred with 160 ml 9.5% ethanol. 56 gm. stearyl alcohol was dissolved in 30 ml ethanol at 70°C . The phosphate was added to the stearyl alcohol solution gradually and with stirring at $55-60^\circ\text{C}$ until relatively dry, then cooled to room temperature with continuous stirring to prevent adherence of phosphate particles. 96 gm cetyl alcohol was dissolved in 20 ml ethanol at $52-55^\circ\text{C}$. The granular phosphate previously coated with stearyl alcohol was then added to cetyl alcohol solution slowly and with continuous stirring and cooled to room temperature. The phosphate remained in small particles, but was screened to remove coarse particles. The coated particles were washed to remove any exposed phosphate. Samples of coated, dried and screened phosphate were tested for solubility at 37°C in distilled water, artificial saliva, gastric juice or intestinal

juice. In testing water solubility, 2 gm coated particles were added to 100 ml H_2O and stirred for periods of 1, 3, 6, 9, 15, 30, 60 and 120 minutes. At end of each time interval the washed sample was filtered and the filtrate was tested for phosphate. A 1-3 minute washing with water removed 10% of the phosphate, while a 30 minute washing removed 45%.

In the above preparation where 400 gm Na_2HPO_4 particles were coated, we obtained a water washed, dry final granular product of 534.8 gm. Our analyses showed that 387 gm of the original phosphate remained. Saliva dissolved somewhat less phosphate from coated particles than did water; while artificial gastric and intestinal juices were somewhat more active. Therefore, it was felt that during the time food was ordinarily in the mouth the phosphate would not have a local effect unless the hamsters stored the food in their cheek pouches for any time.

The Effects of Dietary Sodium Phosphate on Caries

In the experiments done in the summer of 1960, 54 weanling hamsters were used and litter mates of both sexes were divided into 5 groups. (It should be noted that these hamsters were raised at the University by us, and were the off-spring of 2 pregnant female and one male hamster which fortuitously came to the campus in a suitcase of the grantee after a long train trip.) The first group of 14 animals received the basic cariogenic diet of Nizel and Harris used last year also

whole corn	47%
sucrose	14%
liver powder	2%
powdered milk	30%
alfalfa meal	6%
NaCl	1%

Phosphorus content of this diet = 0.393% or (1 X P).

Groups II, III, IV and V consisted of 10 weanling hamsters.

Group II Diet = Group I diet + 0.393% $\text{P/as Na}_2\text{HPO}_4$ (2 x P).

Group III Diet = Group I diet + 2 x 0.393% $\text{P/as Na}_2\text{HPO}_4$ (3 x P).

Group IV Diet = Diet II (2 x P) but Na_2HPO_4 particles coated.

Group V Diet = Diet III (3 x P) but Na_2HPO_4 particles coated.

The animals subsisted on these dietary regimes for 10 weeks. They were fasted for 24 hours. Salivary pH and P estimations were obtained. These represented the termination of time available for the experiment, and because of the building alterations and moving, this part of the experiment was not successful. However, the heads of the animals were removed after a lethal dose of nembutal had been injected and preserved for about 2 months in ethyl alcohol. The jaws were subsequently dissected out, cleaned of extraneous material and stored in 10% formulin. Caries scoring on these jaws proved unusually difficult, and is in progress at this time (3).

Note This experiment could very profitably be repeated next summer.

A simpler experiment (4) was carried out with 3 groups of hamsters (6 in each group) maintained for one week on diets I,

(cariogenic), III (3 x P, uncoated) and V (3 x P, coated). They were then fasted for 24 hours, the mouths and pouches were swabbed with small cotton swabs. The change in weight of the swabs gave the amount of saliva taken up by the cotton. The swabs were covered with 10% trichloroacetic acid in conical, graduated centrifuge tubes. After centrifugation the supernatant liquid was used for determination of the phosphorus by the method of Fiske and Subbarow. These same animals were fed within an hour after the first swabbing. They were allowed to eat for 4 minutes, then 4 minutes after food was removed from them, their mouths were swabbed again and salivary P was determined once more.

Results are given in Table I. Diets No. I, II and III here are Diets I, V, and III in the 10 week experiment. The blanks in the Table represent actual experimental failure, but are recorded as they happened (3).

It should be noted, however, that this experiment was done twice with the same general trend as in Table I and this increases the significance of our results.

Suggested for Next Year

It is these results which suggest the repetition of the longer term experiment. It should be organized to have the mothers on the synthetic diet shortly after the young are born, say at age 7 days.

TABLE 1

PHOSPHORUS CONCENTRATION IN SALIVA OF HAMSTERS
(expressed as mg./gm.)

DIET	ANIMAL NO.	FASTED 24 HRS.	FASTED AND FED	REMARKS
<u>Number I</u> Cariogenic (1 x P)	21	-	-	
	22	-	.071	
	23	-	.040	No significant
	24	.072	.062	local effect
	25	.046	.109	
	26	.238	.184	
<u>Number II</u> Coated Na_2HPO_4 + Diet I (3 x P)	27	.255	.216	
	28	.134	.090	Local and systemic
	29	.191	.620	effect both
	30	.245	.388	significant
	31	.194	.314	
	32	.200	.834	
<u>Number III</u> Uncoated Na_2HPO_4 + Diet I (3 x P)	33	.169	.143	Local effect not
	34	.144	.105	significant.
	35	.043	.101	Systemic effect
	36	-	.285	borders on
	37	.196	.169	significance.
	38	.178	.188	

Experiment II

Inhibition of lactic acid production by addition of phosphates to suspensions of Streptococcus lactis and Lactobacillus casei (4) .

It was decided to do some experiments with oral bacteria in Vitro, in order to obtain further elucidation of the local and/or systemic effect of phosphate on teeth.

Cultures of Streptococcus lactis and Lactobacillus casei were obtained from the Department of Dairy Science, University of Alberta. Actively growing cultures were used to inoculate a tomato juice medium. Bacteria obtained in this way were, thanks to the kindness of the Defence Research Board (Suffield Experimental Station, Ralston), used to inoculate solid agar medium. The solid medium was in stainless steel trays, 24" x 18" by 2 ", covered with stainless steel lids. After the growth period of 24 hours at 37°C, Krebs-Ringer-bicarbonate solution was poured into each tray (about 100 ml.) and small glass beads were also added. By moving a tray to permit the beads to roll along the surface of the solid medium, the bacteria were dislodged, and in suspension were poured out by a vent on the side of the tray. (Note - Thanks are due to Suffield Experimental Station for the loan of trays; we have had similar ones made for us.) The bacteria were centrifuged, washed with more solution, re-centrifuged and the wet packs of the two strains were stored in the refrigerator under nitrogen. The one harvest sufficed for the summer's experiments, by Mr. L.J. Heppler.

One per cent suspensions of bacteria in Krebs-Ringer-bicarbonate solution were used for study of lactic acid production. Glucose was added to 0.3% and the total reaction mixture of 3.5 ml. at pH 7.4, was placed in a Warburg reaction flask. At first conditions were established for steady lactic acid production for one hour by both strains of bacteria. For example, glucose in the reaction mixture at a concentration of 0.3% was optimal. The vessels were gassed for 15 minutes with 5% CO_2 - 95% N_2 . Lactic acid production was estimated by the manometric estimation of CO_2 evolved from the bicarbonate buffer. The various phosphates, used to estimate the effect on lactic acid production, were added

in 0.5 ml. from side arms of the reaction vessel. Phosphorus concentrations in suspensions corresponded to concentrations in the diets used in Experiment I, (0.4%, 0.8%, and 1.2%).

The Krebs-Ringer-bicarbonate solution contained 0.9% NaCl, 1.15% KCl, 1.22% CaCl_2 , 3.82% $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ and 1.3% NaHCO_3 . Reaction mixture = 1% bacterial suspension, 1 ml. K.R.B. solution, glucose and H_2O to 3.0 ml.

The reaction vessels were agitated 64 times per minute and manometer readings were made every 5 minutes. Viability counts on bacteria were made periodically and bacterial concentrations established in suspensions by turbidity measurements. Lactic acid was tested for periodically, colorimetrically, in Warburg flask contents. The pH of the inhibitor phosphates varied over a range of 2-12 and were adjusted to give the final reaction mixture a pH of 7.4.

Figure 1 illustrates results in reaction vessel with Na_2HPO_4 and *Lactobacillus casei*, corresponding to dietary concentrations of 1 x P, 2 x P and 3P. The control flask indicated a steady production of CO_2 by lactic acid. If P corresponding to 1 x P, 2 x P, 3 x P were added at time zero, a moderate inhibition occurred, followed by recovery. If, and this was the rule, P was added from the side-arm of the vessel after a well-established acid production of 30 minutes, an immediate, precipitous fall of CO_2 evolution occurred which involved rapid manipulation of the manometer fluid controls. There followed a trend to recover CO_2 production (broken lines).

Figure 1

Figure 2 illustrates the results with $(\text{NH}_4)_2\text{SO}_4$, a less efficient inhibitor of CO_2 production, and the definite trend to rapid recovery after it was added at 30 minutes for all three concentrations.

Figure 2

Figure 3 illustrates the effect of addition of various P compounds 30 minutes after the steady state was obtained. It gives a visual illustration of the efficiency of various Phosphate compounds added at 2 x P (.786%) concentration in reaction mixture

Figure 3

LACTOBACILLUS GACCI

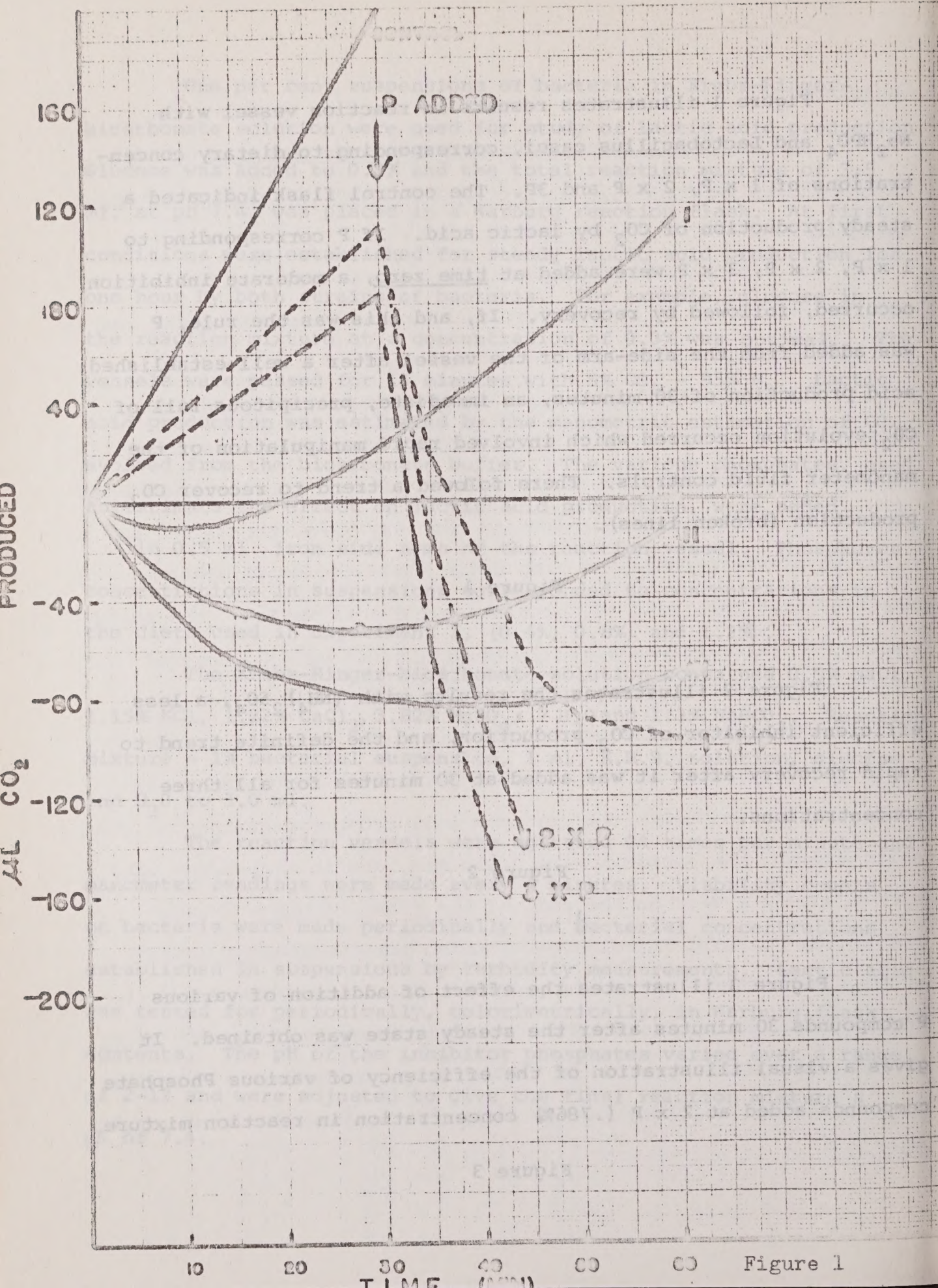
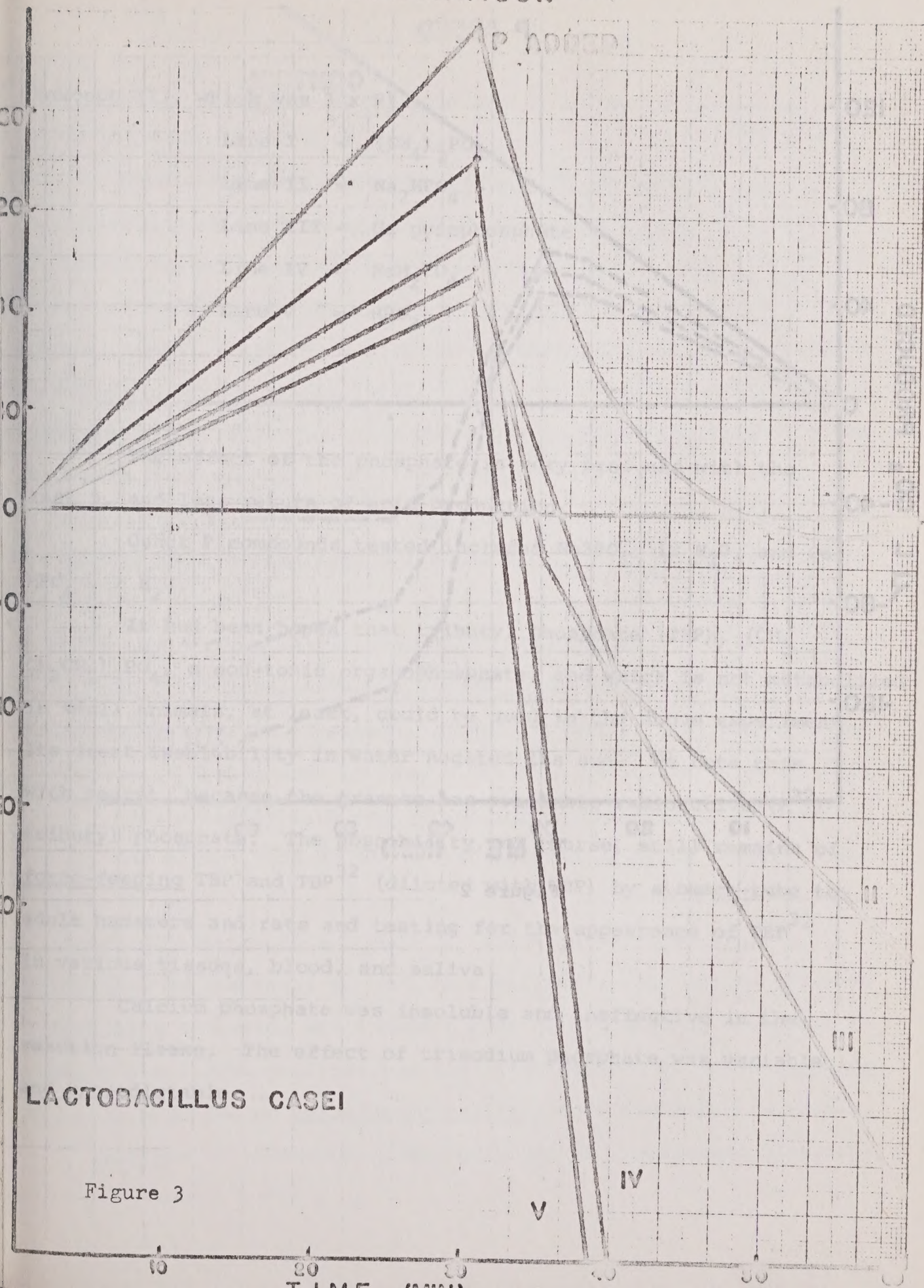


Figure 1



LACTODACILLUS CASEI

P ADDED

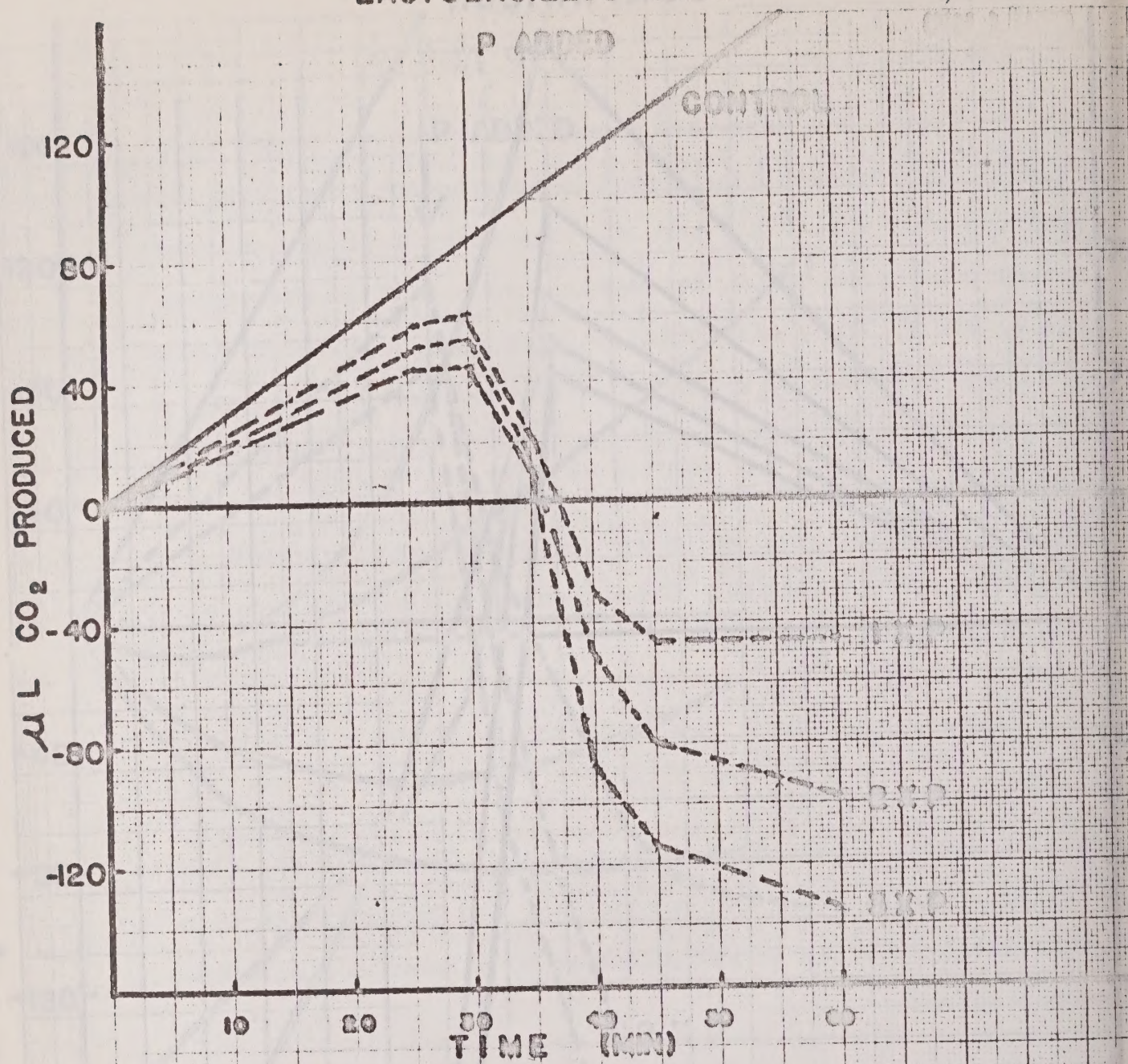


Figure 2

(except III, which was 3 x P).

Line I = $(\text{NH}_4)_2\text{PO}_4$

Line II = Na_2HPO_4

Line III = Na pyrophosphate

Line IV = NaH_2PO_4

Line V = HPO_3

The effect of the phosphate is very profound with the last 3, and less return of acid production.

Other P compounds tested included Na_3PO_4 , $12 \text{ H}_2\text{O}$, and Ca.

$\text{HPO}_4 \cdot 2 \text{ H}_2\text{O}$.

It had been hoped that tributyl phosphate (TBP), $(\text{CH}_3\text{CH}_2\text{CH}_2)_3\text{PO}_4$, a non-toxic organophosphate, and which is not metabolized in small animals, at least, could be used in the above experiment. Its great insolubility in water negated its use. We note this with regret, because the grantee has available radioactive (P^{32}) tributyl phosphate. The possibility, of course, still remains of force-feeding TBP and TBP^{32} (diluted with TBP) by stomach-tube to adult hamsters and rats and testing for the appearance of TBP^{32} in various tissues, blood, and saliva.

Calcium phosphate was insoluble and ineffective in the reaction flasks. The effect of trisodium phosphate was variable and unpredictable.

Conclusions

1. Dietary Na_2HPO_4 appears to have both local and systemic effects. The dietary experiments need to be repeated, not crowded into a summer, but started with younger animals, and with a technician available at all times, to test changes in salivary P and Ca, for trace elements, and correlate these with blood, tissue and tooth analyses. The salivary enzyme, phosphatase, and its kinetics should be studied.

2. Inhibition of CO_2 production by addition of P compounds to bacterial suspensions, appears to be due to decreased lactic acid production. This would indicate oral protection by dietary P compounds.

One could assume that Phosphates in the saliva have altered the bacterial environment to inhibit by buffering with Na ion. However, the result above with HPO_3 indicates that it is not Na ion alone that is effective. It could well be that this P effect is mediated by competitive inhibition in the microorganisms used. (Such studies could be carried out in the pH-Stat now in the grantee's laboratory.)

It is reported by Price (5) that the phosphomonoesterase activity of the algae, *Euglena gracilis*, is repressed by addition of inorganic phosphate. In this instance the enzyme is acid phosphatase, while in *Escherichia Coli* and *Bacillus subtilus* similar results was obtained with alkaline phosphatase on addition of inorganic phosphate. In our experiments somewhere we may have

influenced an enzyme(s) whose decreased activity is reflected in a decreased lactic acid production.

The degree of inhibition and the length of time this persists, in our experiments depends on the phosphate used. One could assume, so far, that a similar local effect could be produced in the mouth by use of proper phosphate to reduce tooth decay.

Experiment III with human teeth, in Addendum to Major Grant Equipment application.

Experiment IV. The establishment of a colony of Harvard Caries Susceptible rats.

Experiment V. Supplying hamsters on special diets for study of morphological changes in Faculty of Dentistry.

Also in Addendum to Major Grant Equipment Application

REFERENCES

- (1) Tuba, J., Heppler, L.J. and Castaldi, C.R., Journal of Dental Research, 40: 654, 1961.
- (2) Nizel, A.E., Harris, R.S., and Parker S.M., Journal of Dental Research, 40: 660, 1961.
- (3) Tuba, J., Heppler, L.J. and Castaldi, C.R., presented at Western Regional Meetings of National Research Council at Suffield Experimental Station, Ralston, Alberta, January, 1962.
- (4) Tuba, J., Heppler, L.J. and Castaldi, C.R., presented at Canadian Conference on Dental Research, Toronto, October 30, 1961.
- (5) Price, C.A., Science, 135: p. 46, 1962.

Also in Addendum to Major Grant Equipment Application

8-1

University of Toronto
FACULTY OF DENTISTRY

124 Edward St.
Toronto 2, Canada

Appendix S

Special Report by December 11, 1961

Dr. J. G. Dale
Mr. G. G. Sheldrick
Award Officer
National Research Council
of Canada
Ottawa 2, Ontario

Dear Sir:

Enclosed is a copy of the final report of the work completed by me during tenure of my Graduate Dental Research Fellowship: 1958-1959, 1959-1960, and 1960-1961.

I am grateful to the National Research Council for the assistance they have given to me during the past three years.

With many thanks, I remain,

Sincerely yours,

Jack G. Dale

JGD:nt
Enclosure

SS-1

University of Toronto
FACULTY OF DENTISTRY

124 EDWARD ST.,

TORONTO 2, CANADA

September 11, 1961

Mr. C. G. Sheldrick
Awards Officer
National Research Council
of Canada
Ottawa 2, Ontario

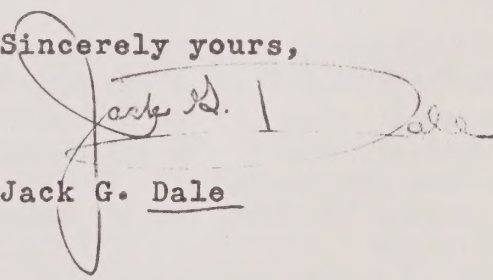
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Sincerely yours,


Jack G. Dale

JGD:nt
Enclosure

S-2

FORSYTH DENTAL INFIRMARY

HARVARD SCHOOL OF DENTAL MEDICINE

140 THE FENWAY

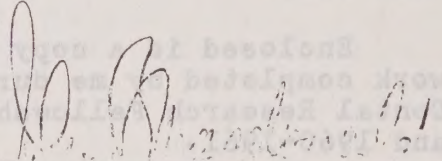
BOSTON 15, MASSACHUSETTS

OFFICE OF THE DIRECTOR

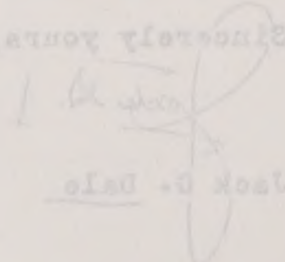
November 21, 1960

To Whom it May Concern:

This is to state that the accompanying document is a true record of the program in graduate training undertaken by Dr. Jack G. Dale at the Harvard School of Dental Medicine and the Forsyth Dental Infirmary.


John B. Macdonald, Director of
Postdoctoral Studies, Harvard School of
Dental Medicine
Director, Forsyth Dental Infirmary

JBW:wrf
enc.


Jack G. Dale

PREFACE

The Harvard-Forsyth Postdoctoral Training Program has a three-fold purpose: (1) to give the graduate student experience and instruction in original investigation of clinical and laboratory problems, (2) to provide intensive training in the care of patients in a clinical specialty, and (3) to furnish opportunities for developing the art and science of imparting knowledge both in clinical and basic science subjects.

HARVARD POSTDOCTORAL PROGRAM

HARVARD SCHOOL OF DENTAL MEDICINE
FORSYTH DENTAL INFIRMARY

Progress Report of Graduate Training

Jack G. Dale, B.A., D.D.S.

Research Fellow in Orthodontics
Tutor in the Basic Medical Sciences

The following progress report includes a detailed account of activities throughout the first two years of the Harvard Postdoctoral Program (September 1958 - September 1960), as well as an estimate for the final year (September 1960 - June 1961).

The report has been organized as follows:

1. Research
2. Patient Care
3. Teaching
4. Formal Presentations
5. Publication
6. Meetings
7. Courses, lectures, seminars, conferences, demonstrations
8. Time-table
9. Summary
10. Appendix

RESEARCH

A method of autogenous bone transplantation utilizing the Millipore filter diffusion chamber has been developed, and a series of experiments designed to study (1) the mechanism of bone resorption, (2) its stimulation, (3) control, and (4) its relationship with the cellular elements of the body have been conducted. Special interest centers on (1) the function, (2) origin, (3) fate, and (4) life span of the associated multinuclear giant cells.

A. Twelve transplantation experiments have been completed on Holtzman albino rats utilizing (1) normal dead scapula, (2) normal dead decalcified scapula, (3) normal live scapula, (4) rachitic dead scapula (diet no. 1.), (5) rachitic dead scapula (diet no. 2.), (6) rachitic live scapula (diet no. 1.), (7) rachitic live scapula (diet no. 2.), (8) normal dead tooth structure, (9) normal dead decalcified tooth structure, (10) normal live

scapular muscle, (11) normal dead scapular muscle, and (12) agar-agar reversible hydrocolloid.

The purpose of these experiments was to study the various conditions under which multinucleated giant cells appear in the tissues. It was our intention to include: (1) living bone, having all its components; (2) dead bone, lacking only vital cells; (3) decalcified bone, lacking the inorganic portion after decalcification; (4) osteoid, lacking the inorganic portion without decalcification; (5) dead tooth structure, lacking only vital cells; (6) decalcified tooth structure, lacking the inorganic portion after decalcification; (7) living and dead tissue other than bone, and finally (8) a foreign body.

Interesting preliminary results have been obtained, however, most of the experiments are in the histological preparation stage.

B. Three transplantation experiments associated with the Millipore diffusion chambers have been conducted to study, particularly, the relationship of cellular elements of the body with bone resorption.

In these experiments a portion of the right scapula was removed, frozen and thawed to kill all associated cells, and divided into three equal parts: (1) one to be histologically prepared immediately and used as a reference section, (2) one to be placed free in the subcutaneous tissue of the left caudal, dorsal, lateral aspect of the same animal as a control, and (3) one to be sealed within a Millipore filter diffusion chamber and placed in a similar area on the right side of the animal.

The latter transplant represents a piece of "cell free" bone within a capsule with pores that allows tissue fluid to enter but not cells.

Bone resorption did occur and many multinucleated giant cells did appear with the "free" transplant. Preliminary results suggest that resorption may occur also within the diffusion chambers without the collaboration of any cells.

Additional experiments utilizing parathyroid hormone, radioactive isotopes, electron microscopy and tissue culture (both in vitro and in vivo) are being considered for further study.

PATIENT CARE

During the past two years, treatment has been carried out on 43 patients (29 new and 14 transfers). Twenty-two patients have required clinical conferences.* At the present time there are 26 active patients (12 nearing retention), 8 completed (in retention), 2 under observation, and 7 discontinued (moving out of state primarily).

*See Appendix

A. The 43 patients may be subdivided, with respect to Angle's classification of malocclusion, into: Class I - 19, Class II division 1 - 16, Class II division 1 subdivision - 2, Class II division 2 - 2, and Class III - 4.

The above malocclusions have also involved: anterior open bite - 5, posterior open bite - 1, closed bite (varying from mild to severe) - 25, anterior crossbite - 11, posterior crossbite - 11, impacted maxillary cuspids - 1, partially impacted maxillary cuspids - 2, congenitally missing teeth - 5, frenectomy - 3, transposed maxillary permanent cuspid - 1, inlocking (maxillary arch completely in facial version to mandibular arch) - 1 and gross asymmetry (Rhombberg's syndrome-hemiatrophy, or unilateral congenital hypoplasia) - 1.

B. Extractions may be subdivided as follows: four bicuspid - 16, two maxillary bicuspid - 3, four first molars - 1, serial - 5, and miscellaneous - 4. Ten patients have required extraction of primary teeth. Interproximal reduction of tooth substance has been carried out on six patients.

C. Mechanotherapy may be subdivided as follows: Edgewise - 12, twin-wire - 12, Begg - 7, monoblock - 6, maxillary lingual arch - 7, mandibular lingual arch - 16, maxillary labial arch - 3, sectional arches - 6, crossbite elastics - 8, positioner - 4, removable appliances - 14, Arnold appliance - 1, chin cap - 1, and retainers - 14.

Headgears have been used in 46 instances: 30 Oppenheim (10 associated with anterior teeth, 20 with the posterior), 4 cervicle, and 12 Northwest.

D. Instruction has been provided by two part-time and three full-time Harvard-Forsyth staff members. Additional instruction is provided by Dr. Moorrees, the Chief of the Orthodontic Department, and visiting clinicians, technicians, and photographers.

It is difficult to make an approximations of activities for the final year, except that I have aspirations of completing treatment on all patients, and making data collections of each child for private use. In addition, I intend to analyze and evaluate the treatment of each individual.

TEACHING

Teaching experience throughout the three years of the program may be subdivided as follows:

A. HARVARD POSTDOCTORAL SEMINARS

1. 1958-59

- a. Mechanism of bone resorption: A Review.
- b. Biology of tooth movement: A Review.

2. 1959-60

- a. Bone transplantation as an experimental method.

3. 1960-61

- a. Bone resorption experimentation.
- b. The influence of papain on endochondral ossification.

B. HARVARD SCHOOL OF DENTAL MEDICINE

1. 1960-61

- a. Occlusion:
One lecture on "Tooth eruption" to the third and fourth year dental students, graduate students, and Forsyth Fellows.
- b. Basic medical sciences:
One tutorial per week for approximately 30 weeks to the first year dental students.

C. HARVARD MEDICAL SCHOOL

1. 1958-59

- a. Histology:
Demonstrator in oral histology to first year medical and dental students.

2. 1960-61

- a. Histology:
To be determined.

D. FORSYTH DENTAL INFIRMARY - SEMINAR PROGRAM

1. 1958-59

- a. Histological evaluation of pulpotomy and pulp capping.
- b. Emergency treatment of fractured anterior teeth.

2. 1959-60

- a. Histological analysis of the temporomandibular joint.

3. 1960-61

- To be determined.

E. FORSYTH SCHOOL FOR DENTAL HYGIENISTS

1. 1958-59

- a. Functional Dental Anatomy:
Three lectures on the "Growth and Development of the Cranio-facial Skeleton."
- b. Histology:
One lecture on the "Histology of Salivary Glands."

2. 1959-60

- a. Oral Embryology and Microscopic Anatomy:
Four lectures on, (a) Embryonic development of the face and

oral cavity, (b) Introduction to histology, (c) Tooth development, and (d) Tooth eruption and shedding.

Two demonstrations on (a) Histologic technique and (b) Blood smears.

3. 1960-61

- a. Oral Embryology and Microscopic Anatomy:
A possible increase in the number of lectures and demonstrations over 1959-60.

F. ORTHODONTIC DEPARTMENT

1. 1958-59

- a. Clinical Diagnostic Conferences:
Eight patient analyses presented.
- b. Treatment Planning Conferences:
Ten patients presented.

2. 1959-60

- a. Clinical Diagnostic Conferences:
Four patient analyses presented.
- b. Treatment Planning Conferences:
Seven patients presented.

3. 1960-61

- a. Clinical Diagnostic Conferences and Treatment Planning Conferences:
To be determined.

4. 1958-60

- a. Each orthodontic fellow has a dental hygienist assisting, at all times, while he is treating patients. It is his responsibility to learn how to utilize auxiliary personnel, and at the same time, to help educate the hygienist.
- b. Each senior orthodontic fellow is obliged to serve as consultant to junior orthodontic fellows and to clinical fellows in the general clinic.

FORMAL PRESENTATIONS

Throughout the past two years, the following clinics and scientific papers have been presented.

A. MASSACHUSETTS DENTAL SOCIETY

1. 1958-59 - Boston

- a. Demonstration: Forsyth Dental Infirmary - Research, Teaching, Patient Care.

2. 1959-60 - Boston

- a. Table clinic: Molar Band Fabrication.

B. HARVARD DENTAL ALUMNI ASSOCIATION

1. 1959-60 - Boston

- a. Symposium: Bone transplantation, bone resorption, and multinucleated giant cells.

The final year presentations which have been scheduled are as follows:

A. NORTHEASTERN SOCIETY OF ORTHODONTISTS - Boston

Table clinic on "Bone resorption and associated multinucleated giant cells."

B. FORSYTH SCHOOL FOR DENTAL HYGIENISTS

Guest speaker for the capping ceremony at Tufts University chapel.
Subject: "The Hygienist and the Dental Profession."

In addition, results are being accumulated and scientific papers prepared for probable presentation at the following meetings:

A. INTERNATIONAL ASSOCIATION FOR DENTAL RESEARCH - Boston

- a. Bone resorption in association with Millipore filter diffusion chambers.
b. Multinucleated giant cells.

B. AMERICAN ASSOCIATION OF ORTHODONTISTS - Denver, Colorado

- a. Bone resorption.

PUBLICATION

Throughout the past two years, the following manuscripts have been prepared:

1. 1958-59

A syllabus for the Forsyth School for Dental Hygienists on "The Growth and Development of the Cranio-facial Skeleton."

2. 1959-60

An article for the Associated Dentists Cooperative Newsletter on "Cardinal Considerations for Graduate Education."

Abstracts of the papers to be presented will be published in the Journal of Dental Research and the American Journal of Orthodontics.

I also contemplate submitting scientific papers on my research to the Journal of Dental Research and the Archives of Oral Biology.

MEETINGS ATTENDED

1. International Association for Dental Research:

a. Boston Chapter

- i 1958-59 - 4
- ii 1959-60 - 6
- iii 1960-61 - to be determined

b. Annual Meeting

- i 1958-59 - San Francisco
- ii 1959-60 - Chicago
- iii 1960-61 - Boston *Member of the local arrangement committee

2. American Association of Orthodontists:

a. Northeastern Section

- i 1959-60 - Hartford, Connecticut
- ii 1960-61 - Boston

b. Annual Meeting

- i 1959-60 - Washington, D.C.
- ii 1960-61 - Denver, Colorado

3. Massachusetts Dental Society

a. Annual Meeting

- i 1958-59 - Boston
- ii 1959-60 - Boston
- iii 1960-61 - Boston

4. New England Dental Society

a. Annual Meeting

- i 1958-59 - Boston
- ii 1959-60 - Boston
- iii 1960-61 - Boston

5. First Annual Margolis Lecture Series - June 12, 1959

6. Harvard Medical Society - December 7, 1960

7. Harvard Dental Alumni - October 6, 1960

8. Howard Mueller Memorial Lecture
 - i John Enders - Nobel Prize winner in Medicine
 - ii Joshua Lederberg - Nobel Prize winner in Medicine
9. National Academy of Sciences, National Research Council - March 16, 1960, Chicago
10. International Conference on Oral Biology - September 7-8, 1960, New York
11. International Conference on Advanced Education - September 9, 1960, Boston
12. New York Academy of Sciences - "Oral Metabolism" - October 15-17, 1960, New York
13. Gordon Research Conference - July 11-15, 1960, Meridan, New Hampshire
14. American Association of Dental Schools - 1961, Boston

[illegible]

	Forsyth Seminars*	Harvard School of Dental Medicine	Harvard Medical School	Harvard School of Public Health	Harvard Senior Seminars	Harvard Medical Society Seminars	I.A.D.R. Seminars	Postdoctoral Seminars*	Orthodontic Seminars*	Orthodontic Diagnostic Conferences*	Orthodontic Treatment Conferences*	Demonstrations & Typodont	Total 1958-60	1960-61
History	1												1	
Research	10												10	6
Orthodontic patient analysis									176 36				212	70
Orthodontic treatment planning											43 12		55	15
Biomechanical principles														
(a) Edgewise													125	125
(b) Begg													65	65
(c) Twin													50	50
(d) Monoblock													10	10
(e) Headgear													4	4
(f) Miscellaneous													15	15
Total													269	56
Orthodontic materials and laboratory demonstrations (including indirect band fabrication)													20	5
Photography (orthodontic)													8	2
Total													1404	475

*See Appendix

Time	Monday	Tuesday	Wednesday	Thursday	Friday	Saturday	Sunday
8-9	Research	Teaching F.S.D.H.	Research	Research	Occlusion H.S.D.M.		
9-10	Forsyth Seminar	Courses, H.M.S., H.S.P.H.	Forsyth Seminar	Courses, H.M.S., H.S.P.H.	Forsyth Seminar	Courses H.M.S.	
10-11	Patients (clinic)	Patients (clinic)	Patients (clinic)	Patients (clinic)	Patients (clinic)	Research	
11-12							
12-1	Postdoctoral Seminar	Diagnostic Conference	Orthodontic Seminar	Diagnostic Conference	Treatment Conference		
1-2							
2-3	Patients (clinic)	Courses H.M.S., H.S.P.H.	Patients (clinic)	Courses H.M.S., H.S.P.H.	Clinical Demonstration		Patients (Laboratory)
3-4							
4-5	Patients (Laboratory)		Patients (Laboratory)				Research
5-6							
6-7	Research	Tutorial H.S.D.M.	Research	Research			
7-8							
8-9							
9-10							

*During June, July: one-half time in orthodontic clinic and laboratory, one-half time in research.

*During June, July: one-half time in orthodontic clinic and laboratory, one-half time in research.

SUMMARY

Hours

1. TOTAL TIME

a. Approximately 60 hours per week

1. 1958-59 - 39 weeks (minus June, July, August)	
2. 1959-60 - 39 weeks " " " "	= 7020
3. 1960-61 - 39 weeks " " " "	
<u>117</u>	

b. Approximately 50 hours per week

1. 1958-59 - 12 weeks (June, July, August*)	
2. 1959-60 - 8 weeks (June, July)	= 1200
3. 1960-61 - 4 weeks (June)	
<u>24</u>	
	<u>8220</u>

*Full-time conducting research - August 1958

2. PATIENT CARE

a. Approximately 15 hours per week (patients-clinic)

15 x 117	1735
16 x 20 (summer)	<u>320</u>
	2055

b. Approximately 6 hours per week (lab-appliances, models, etc.)

6 x 117	702
5 x 20 (summer)	<u>100</u>
	802

2857

3. RESEARCH

a. Approximately 15 hours per week

15 x 117	1735
----------	------

b. Approximately 20 hours per week (summer)

20 x 20	400
---------	-----

c. Approximately 40 hours (August 1958)

4 x 40	160
--------	-----

2295

4. INSTRUCTION (Courses, lectures, seminars, etc.) Hours

Approximately average 16 hours per week

16 x 117 1872

Summer (June, July, August) - minimal 7

1879

5. TEACHING

	1958-59	1959-60	Estimate 1960-61
--	---------	---------	---------------------

Teaching time 20 13 50 83

Preparation time 204 134 304 642

725

6. MEETING PRESENTATIONS

Presentation time 3 2.25 4.75 10

Preparation time 3 25 132 160

170

7. MANUSCRIPTS

50 25 100 175

8. TOTAL 8101

Time not accounted for e.g., traveling, etc. 119

8220

APPENDIX

Orthodontic Diagnostic Conference

Orthodontic patients are generally required to have clinical conferences according to the following procedures:

1. Data are collected by the orthodontic fellow: Study models, cephalograms, wrist and hand roentgenograms, "full-mouth" intra-oral roentgenograms, intra-oral color pictures, black and white portraits, somatotype photographs, a complete dental and orthodontic examination, and a complete history.
2. Data are analyzed and a detailed report is prepared. The cephalometric analyses consist of a Moorrees Mesh and one other method. A different analysis for each patient is encouraged for the second analysis. Physiologic maturation is determined statistically by three different analyses: (1) bone development (Greulich and Pyle); (2) tooth calcification (Fanning); and (3) tooth emergence (Hurme).
3. A physical examination is conducted by the pediatrician, and a report is prepared. The orthodontic fellow is in attendance during the examination.
4. The parents and patient have interviews with the psychiatrist, and a report is prepared.
5. The nutritionist examines the diet of the patient, and prepares a report.
6. The somatotype photographs are examined by the physical anthropologist, and a statement is prepared.
7. Consultations are frequently carried out with the general dentist, periodontist, and oral surgeon, and reports are prepared.
8. All reports are presented and discussed at the conference with all orthodontic staff and fellows present.
9. On the basis of this conference, the etiology, therapeutic modifiability, indications and contra-indications for treatment are determined. Thus, emphasis is placed on a comprehensive diagnosis and prognosis. If the patient requires treatment in areas other than orthodontics, referrals, or further consultations are arranged.

Orthodontic Treatment Conferences

If treatment is indicated as a result of the diagnostic conference, treatment planning is discussed by the orthodontic staff and fellows from several points of view.

When the treatment has been decided upon, a full report of both conferences is typed into the patient's record, and treatment is then initiated according to the plan advised.

Patient treatment progress is reviewed from time to time in the treatment conferences. In addition, once a year, the treatment progress of the patients of each fellow are reviewed in the clinic by the complete orthodontic staff.

Orthodontic Seminars

Each week, review of the literature seminars are held in the Orthodontic Department.

The subject, and two or three references to be thoroughly read by all fellows and staff, are decided upon one week in advance.

During the seminar, Dr. Moorrees conducts a very informal discussion on the relative merits and shortcomings of the articles, and on the information which the authors are attempting to convey. The fellows are encouraged to criticize freely and constructively, and perhaps to advance original ideas for further study and investigation.

Postdoctoral Seminars

The postdoctoral seminars are held each Monday from 12:00 noon until 1:45 p.m. The time is divided as follows: (1) Staff-fellow luncheon - 30 minutes, (2) Formal presentation - 45 minutes, and (3) Discussion period - 30 minutes.

Scientific material is presented, by the postdoctoral fellows, to research and clinical faculty members of the Forsyth Dental Infirmary and the Harvard School of Dental Medicine.

Each fellow is required to make two presentations per year: (1) A progress report of his research project and (2) A review of an area of interest related to his research.

Each paper is discussed and evaluated thoroughly by the staff and fellows. Constructive criticism is offered for the purpose of improving the calibre of research and scientific presentation.

Forsyth Seminars

The Forsyth seminars are conducted each day from 9:00 a.m. until 10:00 a.m. They consist of a forty-five minute formal presentation and a fifteen minute discussion period.

Material is presented by faculty members from Harvard University, Tufts University, Boston University, Brandeis University, Massachusetts Institute of Technology, and associated institutions and hospitals. The research and clinical fellows are given an opportunity to participate in the program.

Frequently, special lectures are given by research scientists, clinicians, and teachers from institutions in other areas of the United States, and other countries of the world.

SUPPLEMENT

Page 6 FORMAL PRESENTATIONS

C. MASSACHUSETTS DENTAL HYGIENISTS' ASSOCIATION

Guest speaker for annual meeting at Statler Hilton Hotel.

Subject: "Current concepts of orthodontics and their importance to the dental hygienist".

A. INTERNATIONAL ASSOCIATION FOR DENTAL RESEARCH

Presentation: "The use of diffusion chambers in bone resorption studies", at annual meeting, Boston

*Named Winner of Edward Hatton Award.

Page 7 PUBLICATIONS

3. 1960-61

The use of diffusion chambers in bone resorption studies. J. D. Res. 40:674, July-August, 1961. Abs.

APPENDIX T

Members of the Associate Committee on Dental Research and its Executive, with their terms of appointments

	<u>Term to expire</u>
*1. Dr. K. J. Paynter, <u>Chairman</u> Division of Dental Research, University of Toronto, Toronto, Ontario.	31 March 1962
2. Dr. E. W. R. Steacie, <u>ex officio</u> , President, National Research Council, Ottawa 2, Ontario.	- - - -
3. Dr. R. F. Farquharson, <u>ex officio</u> , Vice-President (Medical), National Research Council, Ottawa 2, Ontario.	- - - -
4. Dr. D. L. Thomson, <u>ex officio</u> , Vice-Principal, McGill University, Montreal, P.Q.	- - - -

Representing the Dental Profession

5. Dr. C. Castaldi, Faculty of Dentistry, University of Alberta, Edmonton, Alberta.	31 March 1963
6. Dr. L. E. Francis, Department of Clinical Dentistry, McGill University, Montreal, P.Q.	31 March 1962
*7. Dr. I. Kleinberg, Department of Biochemistry, University of Manitoba, Winnipeg, Manitoba.	31 March 1962
8. Dr. J. W. Neilson, Dean, Faculty of Dentistry, University of Manitoba, Winnipeg, Manitoba.	31 March 1962

* Member of the Executive

Term to expire

9. Dr. G. Nikiforuk,
Division of Dental Research,
University of Toronto,
Toronto, Ontario.

31 March 1963

Representing the Royal Canadian Dental Corps,
Department of National Defence

10. Brig. K.M. Baird, ex officio,
Director General of Dental Service,
Royal Canadian Dental Corps,
Department of National Defence,
Ottawa, Ontario.

- - - -

Representing the Division of Dental Health,
Department of National Health and Welfare

11. Dr. H.K. Brown, ex officio,
Chief, Dental Health Division,
Department of National Health and Welfare,
Ottawa, Ontario.

- - - -

Representing Dental Research,
Department of Veterans Affairs

12. Dr. L.A. Kilburn, ex officio,
Director of Dental Research,
Department of Veterans Affairs,
Ottawa, Ontario.

- - - -

Representing the Basic and Medical Sciences

13. Dr. L.F. Belanger,
Department of Histology,
University of Ottawa,
Ottawa, Ontario.

31 March 1962

- *14. Dr. J.P. Lussier,
Faculty of Dentistry,
University of Montreal,
Montreal, P.Q.

31 March 1964

Member of the Executive

* Member of the Executive

		<u>Term to expire</u>
*15.	Dr. A.G. Gornall, Department of Pathological Chemistry, University of Toronto, Toronto, Ontario.	31 March 1962
16.	Dr. C.P. Leblond, Department of Anatomy, McGill University, Montreal, P.Q.	31 March 1963
17.	Dr. A.J. Rhodes, School of Hygiene, University of Toronto, Toronto, Ontario.	31 March 1963
18.	Dr. R.L. de C.H. Saunders, Department of Anatomy, Dalhousie University, Halifax, Nova Scotia.	31 March 1964
*19.	Dr. G.D. Scott, Department of Physics, University of Toronto, Toronto, Ontario.	31 March 1963
20.	Mr. A.D. Armstrong, <u>Secretary</u> , National Research Council, Ottawa 2, Ontario.	- - - -

* Member of the Executive

INITIAL DISTRIBUTION

(i) To members of the Associate Committee on Dental Research

- | | |
|--------------------------------------|-------------------------------|
| 1. Dr. K.J. Paynter, <u>Chairman</u> | 11. Dr. C.P. Leblond |
| 2. Brig. K.M. Baird | 12. Dr. J.P. Lussier |
| 3. Dr. L.F. Belanger | 13. Dr. J.W. Neilson |
| 4. Dr. H.K. Brown | 14. Dr. G. Nikiforuk |
| 5. Dr. C. Castaldi | 15. Dr. A.J. Rhodes |
| 6. Dr. R.F. Farquharson | 16. Dr. R.L. de C.H. Saunders |
| 7. Dr. L.E. Francis | 17. Dr. G.D. Scott |
| 8. Dr. A.G. Gornall | 18. Dr. E.W.R. Steacie |
| 9. Dr. L.A. Kilburn | 19. Dr. D.L. Thomson |
| 10. Dr. I. Kleinberg | 20. Mr. A.D. Armstrong |

(ii) To other persons:

21-25 Deans of Dental Schools (who are not members of the Committee)

- | | | |
|---------------|---|-------------------|
| 21. Alberta | - | Dr. H.R. MacLean |
| 22. Dalhousie | - | Dr. J.D. McLean |
| 23. McGill | - | Dr. J. McCutcheon |
| 24. Montreal | - | Dr. P. Geoffrion |
| 25. Toronto | - | Dr. R.G. Ellis |

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NATIONAL RESEARCH COUNCIL OF CANADA

PROCEEDINGS
OF THE
TWENTY-NINTH MEETING
OF THE
ASSOCIATE COMMITTEE ON
DENTAL RESEARCH

OTTAWA

28 FEBRUARY - 1 MARCH 1963



CABLE ADDRESS "RESEARCH"

IN YOUR REPLY PLEASE QUOTE

FILE No.

NATIONAL RESEARCH COUNCIL
CANADA

DIVISION OF ADMINISTRATION
AND AWARDS

OTTAWA 2,

7 May 1963

Dr. R. G. Ellis, Dean,
Faculty of Dentistry,
University of Toronto,
Toronto, Ontario.

UNIVERSITY OF TORONTO
FACULTY OF DENTISTRY
OFFICE OF THE DEAN

RECD. MAY 9 - 1963

Dear Dr. Ellis:

ANSWERED _____
REFERRED TO _____
FILE _____

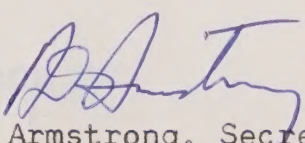
Please make the following adjustments in the minutes of the 29th meeting of the Associate Committee on Dental Research:

- 1) on page 17, item 18, third line, the name of Dr. A. G. Gornall should be changed to read Dr. C. Castaldi.
- 2) On the back page showing the mailing list copy number 25 should be sent to:

Manitoba

Dr. J.W. Neilson

Yours sincerely,


A. D. Armstrong, Secretary,
Associate Committee on Dental Research.

ADA:lr

ASSOCIATE COMMITTEE ON DENTAL RESEARCH

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NATIONAL RESEARCH COUNCIL OF CANADA

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PROCEEDINGS

of the

TWENTY-NINTH MEETING

of the

ASSOCIATE COMMITTEE ON DENTAL RESEARCH

OTTAWA

28 FEBRUARY - 1 MARCH 1963

ASSOCIATE COMMITTEE ON DENTAL RESEARCH

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Dr. L. E. Francis
 Dr. A. G. Gornall
 Dr. R. M. Gwinnager
 Dr. L. A. Kilburn
 Dr. I. Kleinberg
 Dr. S. Wah Leung
 Dr. J. P. Lussier
 Dr. G. Nieldorik
 Dr. M. Poplove
 Dr. A. J. Rhodes
 Dr. R. L. DeC. H. Saunders
 Dr. G. B. Scott
 Dr. D. M. Tanner
 Dr. E. C. Will
 Mr. A. D. Armstrong, Secretary

Regrets at their inability to attend were received from:

Dr. L. F. Belanger
 Dr. G. E. Leblond

The Chairman extended a welcome to the new members of the Committee and to the guests Dr. S. Wah Leung and Dr. R. V. Blackmore. He also introduced Dr. M. Poplove of the Department of National Health and Welfare, and Dr. D. M. Tanner who would shortly be taking Dr. L. A. Kilburn's place as representative for the Department of Veterans Affairs.

NATIONAL RESEARCH COUNCIL

Proceedings

of the

Twenty-Ninth Meeting

of the

ASSOCIATE COMMITTEE ON DENTAL RESEARCH

28 February - 1 March 1963

The first session convened in the Council Chamber of the National Research Council, Ottawa, at 9:30 a.m., 28 February 1963.

1. Attendance

Dr. K. J. Paynter, Chairman

Dr. K.M. Baird

Dr. R.V. Blackmore

Dr. C. Castaldi

Dr. R.F. Farquharson

Dr. L.E. Francis

Dr. A.G. Gornall

Dr. R.M. Grainger

Dr. L.A. Kilburn

Dr. I. Kleinberg

Dr. S. Wah Leung

Dr. J.P. Lussier

Dr. G. Nikiforuk

Dr. M. Poplove

Dr. A.J. Rhodes

Dr. R.L. deC.H. Saunders

Dr. G.D. Scott

Dr. D.M. Tanner

Dr. W.C. Wilt

Mr. A.D. Armstrong, Secretary

Regrets at their inability to attend were received from:

Dr. L.F. Belanger

Dr. C.P. Leblond

The Chairman extended a welcome to the new members of the Committee and to the guests Dr. S. Wah Leung and Dr. R.V. Blackmore. He also introduced Dr. M. Poplove of the Department of National Health and Welfare, and Dr. D.M. Tanner who would shortly be taking Dr. L.A. Kilburn's place as representative for the Department of Veterans Affairs.

2. Minutes of the Twenty-Eighth Meeting

The minutes of the Twenty-Eighth Meeting had been distributed to all members and were therefore taken as read.

It was MOVED by Dr. J. P. Lussier and SECONDED by Dr. G. D. Scott

THAT the Minutes be approved.

CARRIED

3. General Business

The Chairman announced that he had written a letter of sympathy to Mrs. E. W. R. Steacie, wife of the late President, on the death of her husband.

4. Chairman's Remarks

1. Research Support in Canada

This year an estimated $13\frac{1}{4}$ million dollars will be spent on extramural support for medical research by agencies that grant funds for this purpose on a national basis. In addition there will be perhaps another \$3,000,000 provided from other agencies. The total estimated for medical research for 1963 (including dental) is just over \$16,000,000.

Total extramural medical research spending has not increased particularly rapidly over the last three years or so. Most national agencies have not increased their allocation for this purpose appreciably at all. The Medical Research Council is the one major exception. The M. R. C. allocation this year will be about \$5,000,000, - up about 40% over the 3.6 million spent in 1961-62.

Support for dental research through this Committee which is comparable to M. R. C. for medical research, has increased at an even faster rate over the same time. Our spending this year will be some 65-70% greater than 1961-62, and it will be more than double our allocation in 1960-61.

One can readily foresee within the next few years a need for expansion of space in which to do dental research becoming as acute as the present need in medicine. A problem even more pressing in dentistry to-day however, is the need to develop the area of clinical investigation. In medicine some 40-45% of the \$16,000,000 that will support research this year will finance clinical investigations. Only about 10-15% of the total spending on dental research this year will support clinical studies. The need to develop such studies in dentistry is becoming ever more

urgent as governments become more interested in the development of plans whereby dental health services might be distributed more widely.

This lack of research development in the clinical areas of dentistry relates primarily to two factors - the lack of clinicians with an adequate background of training in research methodology, and space in which to carry out clinical studies.

Lack of trained personnel is of course, not confined to the clinical area. The greatest need for research expansion in general is for people with training and ability to carry out projects. Nor is the shortage of research space confined to the clinical areas; it may simply be more acute at the moment. While far more study could be carried out in the clinics of dental schools, the potential of these areas falls far short of requirements. What is needed are large public service clinics to function as hospitals do in other health areas, where patients are not selected as they are in dental school clinics. These should form the centres for greatly needed studies on methods of providing dental services more widely, on effectiveness of preventive procedures, on utilization and integration of services of auxiliaries, etc. It seems to me that until such service clinics do come into existence clinical dental research will continue to fall far short of requirements.

2. General Research Grants to Deans

You will recall last year that this Committee considered the possibility of recommending that each dental school Dean be given a general grant for support of research activities in his school. This has now been going on in medical schools over the last two or three years. Each medical Dean last year received \$12,000 and I understand a similar amount will be granted this year. These grants are very popular with the Deans, and good for research. They create a flexibility in a growing research programme that is impossible where the research is financed solely through the necessarily somewhat confining grants-in-aid.

One of our problems in relation to making such a grant to dental Deans is the fact that the National Research Council now provides each university President with a general research grant to be used at his discretion. The Council is understandably reluctant to make an additional grant to a single department of the university. Perhaps consideration might be given to the idea that some portion of the President's grant might be earmarked for dentistry. The usefulness of such a fund to provide for unexpectedly available staff, for graduate student support, or for equipment or travel, cannot be denied.

3. Availability of Research Money Versus Demand

At the meeting of the Interdepartmental Medical Research Co-ordinating Group in January this year, it was evident that on the average the total requests for research support from each granting agency were running 25-30% higher than the estimated available money. This indicated, at least on the surface, that research funds might be in serious short supply.

The group at that meeting felt that such a "paper deficit" did not in fact reflect an actual shortage. The opinion was expressed that no worthwhile request would likely be denied, and no hardship inflicted. Indeed the closer scrutiny of applications likely to result when demand exceeds supply, might in the long run, be a healthy thing.

4. Personnel Support

The Medical Research Council are now supporting about thirty Associateships in the twelve medical schools in Canada. The Dental Committee is still supporting only one.

You will recall last year that we revised the terms of our Associateships to provide support for the young man who had just completed his graduate training and who needed interim support until he was firmly established in his academic position. This year the Medical Research Council have announced the establishment of Medical Research Council Scholarships which are designed to serve a similar purpose. These will fill the gap between the Associateship and the M. R. C. Fellowship.

The Medical Research Council have also increased the amount awarded to Fellows to provide support for dependents. Applications for travel for amounts up to \$150 will also be considered from M. R. C. Fellows.

At the Interdepartmental Medical Research Co-ordinating Group meeting all members warmly endorsed the principle of support for undergraduate students during the summer in order to provide them with a research experience. This was considered to be one of the best possible recruitment procedures for research personnel.

5. Second Canadian Conference on Dental Research

Last year this Committee provided a sum of \$3,000 to help support the Second Canadian Conference on Dental Research.

The Conference was held at the Banff School of Fine Arts October 9-11, 1962. About forty research workers were in attendance,

and approximately twenty-five scientific papers were presented. Abstracts of the papers have been published in the Journal of the Canadian Dental Association.

During the meeting approval was given to the idea of forming a national Canadian Dental Research Group. Steps are now being taken to bring this about. At the moment it has not yet been decided whether this should form a separate Canadian Association for Dental Research, or whether it would be wiser to form a separate Division of the International Association for Dental Research. Plans will become more definite later this year. At the moment the I.A.D.R. is reviewing its own organization. During the March meeting of the I.A.D.R. discussions will be held with representatives of that organization in relation to the Canadian Research Group.

5. Executive Action 1962-63

(a) Name of Applicant: NIKIFORUK, G.

Request: That Dr. G. Nikiforuk, University of Toronto, be permitted to substitute Mr. G. Baker for Mr. G. Esilman for a Summer Undergraduate Research Assistantship.

Decision: Request granted

(b) Name of Applicant: TUBA, J.

Request: That the Executive Committee review supporting information from Dr. J. Tuba, University of Alberta, for the need for a Cary Model 15 Recording Spectrophotometer.

Decision: The Executive Committee examined the supporting information and confirmed the Committee's decision to award \$4,000.00 to Dr. J. Tuba towards the cost of the machine.
Request granted

(c) Name of Applicant: SAUNDERS, R. L. deC.H.

Request: That Dr. R. L. deC.H. Saunders, Dalhousie University, be provided with \$795.00 to pay a Summer Undergraduate Dental Research Assistant. Dr. Paynter's grant was to be reduced by this much to cover the cost.

Decision: Request granted

(d) Name of Applicant: KASLOFF, Z.

Request: That Dr. Z. Kasloff, University of Manitoba, be permitted to charge an expenditure of \$238.20 against his grant DA-90 to bring Dr. Ralph Phillips of Indiana University to his laboratory.

Decision: \$150.00 granted

(e) Name of Applicant: LEBLOND, C. P.

Request: That Dr. C. P. Leblond, McGill University, be permitted to support a summer assistant for four rather than three months.

Decision: Request not granted. However, grantee could use funds from existing grant for that purpose if necessary.

(f) Name of Applicant: SOLOMONS, C. C.

Request: That Dr. C. C. Solomons, McGill University, be permitted to spend \$166.00 of his grant for travel purposes.

Decision: Request granted

(g) Name of Applicant: KLEINBERG, I.

Request: That Dr. I. Kleinberg, University of Manitoba, be permitted to use funds saved due to his summer assistant, Mr. Kaufman, being able to work only one month instead of two months, to support Mr. Johnson, a first year dental student.

Decision: Request granted

(h) Name of Applicant: PRITCHARD, E. T.

Request: That Dr. E. T. Pritchard, University of Manitoba, be provided with a travel grant for travel from Boston, Mass. to Winnipeg, Manitoba.

Decision: Request granted

(i) Name of Applicant: LEBLOND, C. P.

Request: That Dr. C. P. Leblond, McGill University, be permitted to employ Mr. Mahoney in the place of Mr. Landers as a Summer Undergraduate Research Assistant.

Decision: Request granted

(j) Name of Applicant: LUSSIER, J. P.

Request: That Dr. J. P. Lussier be awarded a supplement of \$300.00 to buy an electric desalter.

Decision: Request granted

It was MOVED by Dr. A. G. Gornall and SECONDED by Dr. I. Kleinberg

THAT Executive Actions taken during 1962-63 be approved.
CARRIED

6. Deans' Support Funds

A general discussion took place concerning general grants to Deans of Dental Schools. After pointing out the need for extra funds caused by rapidly expanding dental schools,

It was MOVED by Dr. A. G. Gornall and SECONDED by Dr. R. L. deC. H. Saunders

THAT the Executive should prepare a case to show the need for making general grants to Deans of Dental Schools for the support of dental research.

CARRIED

7. Summer Undergraduate Dental Research Scholarships

The Committee examined the terminology used in describing the special scholarships awarded to undergraduate dental students after notice had been served by the Council Executive that the terminology was not in line with the Council's policy of not awarding scholarships to undergraduates. Accordingly,

It was MOVED by Dr. L. E. Francis and SECONDED by Dr. I. Kleinberg

THAT the terminology of the award and the administration of its functioning should be changed to conform to Council policy.

CARRIED

8. Financial Support for Dental Research

After careful consideration,

It was MOVED by Dr. R. M. Grainger and SECONDED by Dr. I. Kleinberg

THAT the Executive prepare a special study with a view to increasing financial support by Government and private

sources so that larger grants could be awarded immediately and adequate financial support for long term dental research development support be established.

CARRIED

9. Dental Research Fellowships

After consideration of Medical Research Council practice in this area,

It was MOVED by Dr. J. P. Lussier and SECONDED by Dr. L. E. Francis
THAT \$500.00 be added to each Fellow's stipend for the first child, and \$200.00 for each additional child, to be adopted in 1964-65.

CARRIED

It was MOVED by Dr. C. Castaldi and SECONDED by Dr. A. G. Gornall
THAT the regulations for the Dental Fellowship be changed to enable all graduates with appropriate science qualifications to apply for a Fellowship.

CARRIED

10. Reports from Grantees

Eighteen grantees submitted progress reports on the work on which they had been engaged during 1962-63.

11. Meeting of the Executive

The Chairman informed the Committee that the members of the Executive (Dr. A. G. Gornall, Dr. I. Kleinberg, Dr. J. P. Lussier, Dr. G. D. Scott, in addition to the Chairman) had met on 27 February 1963, at 9:30 a.m. Preliminary consideration had been given to the applications for Associateships, for Fellowships, and for grants in aid; the recommendations of the Executive were submitted to the Committee as each application was reviewed.

12. Financial Status of Committee

The Secretary informed the Committee that up to \$167,000 could be made available to the Committee for 1963-64 for grants, \$25,000 (approximately) for Fellowships, \$9,500 for Associateships, and \$2,000 for Committee Expenses. Of the \$167,000 for grants, \$38,000 was encumbered for term grants previously approved.

13. Applications for Associateships

The salary of the incumbent holding the Dental Research Associateship tenable during 1962-64 was considered. Action was taken as follows:

		<u>Salary</u>	
		1962-63	1963-64
E. T. Pritchard,	1962-63		
Dept. of Oral Biology,		Recommended	Approved
Faculty of Dentistry,	\$8700*	\$9600*	\$9600*
University of Manitoba			

* Plus payment of Applicant's share of cost of pension and other benefits in which university staff members normally participate.

14. Applications for Fellowships

Nine applications for Dental Research Fellowships tenable during 1963-64 were considered: four for renewal of current Fellowships and five for a first award. Action was taken as follows:

Ellis, W. B. To work in the Dept. of Bio-chemistry, University of Toronto, under Dr. C. S. Hanes APPROVED \$3,500

Golub, L. M. To work in the Dept. of Oral Biology, Faculty of Dentistry, University of Manitoba, under Dr. C. M. Dowse NOT APPROVED

Gray, A. S. To work in the Faculty of Dentistry, University of Toronto, under Dr. R. M. Grainger APPROVED \$4,500

Johnson, R. H. To work in the Dept. of Oral Diagnosis and Oral Medicine, University of Indiana, under Dr. D. F. Mitchell APPROVED \$4,000

Johnston, M. C. To work in the Dept. of Dentistry and Dental Research, and Dept. of Anatomy, University of Rochester, under Dr. E. Johansen APPROVED \$5,000

Kaufman, H. W.	To work in the Dept. of Oral Biology, University of Manitoba, under Dr. I. Kleinberg	APPROVED \$4,000
Sandham, H. J.	To work in the Dept. of Oral Biology, Faculty of Dentistry, University of Manitoba, under Dr. I. Kleinberg	APPROVED \$4,500
Taichman, N. S.	To work at the Harvard School of Dental Medicine, under Dr. P. Goldhaber	APPROVED \$5,000
Weinstock, A.	To work at the Harvard School of Dental Medicine, under Dr. J. T. Irving	APPROVED \$4,000

It was MOVED by Dr. A. G. Gornall and SECONDED by Dr. L. E. Francis
 THAT payment of these grants be made as outlined in the above schedule.

CARRIED

15. Applications for Grants, 1963-64

The Chairman noted that funds were already committed for the coming year for Term Grants as follows:

Copp, D. H.	Phosphorus deficiency:	\$6,400
Physiology	Effects on bones and teeth	The third instalment
Br. Columbia	DT-59	of a three-year
		Term Grant
Grainger, R. M.	Statistical Consultation and	\$7,000
Dental Research	Analysis Unit	The third instalment
Toronto	DT-89	of a three-year
		Term Grant
Kleinberg, I.	1. Further studies on the	\$6,600
Biochemistry	role of the dental plaque in	The third instalment
Manitoba	the caries process	of a three-year
	2. Further studies on the	Term Grant
	effect of substances removed	(See supplementary
	during the refining of carbo-	Annual Grant)
	hydrates on calcium phosphate	
	solubility	
	DT-78	

Leblond, C. P. Anatomy McGill	Entry of carbohydrates and proteins into teeth as shown with the help of radioactive elements DT-23	\$5,000 The second instalment of a three-year Term Grant (See supplementary Annual Grant)
Nikiforuk, G. Dental Research Toronto	Organic compounds of dental tissue DT-84	\$5,000 The third instalment of a three-year Term Grant
Paynter, K. J. Dental Research Toronto	Factors influencing tooth morphology and caries susceptibility DT-72	\$8,000 The second instalment of a three-year Term Grant

Thirty-three grant applications were then considered. Many of them had been reviewed before the meeting by scientists qualified in the particular field involved, and copies of the reviewers' comments were made available to all members of the Committee.

<u>Applicant</u>	<u>Title and Amount Requested</u>	<u>Recommendation</u>
Anderson, D.L. Dental Research Toronto	Histochemical investigation of cell cytoplasm and inter-cellular interfibrillar material DA-86 \$1,000	APPROVED \$1,000
Baril, C. Orthodontics Montreal	Electromyography of Vth cranial nerve muscles DA-107 \$5,355	APPROVED \$5,000
Blackmore, R. V. Dental Research Alberta	Factors influencing the propagation of <u>herpesvirus hominis</u> in animal cell cultures and in experimental animal infections DA-102 \$7,800	APPROVED \$7,300
Dale, J. G. Dental Research Toronto	The study of bone resorption by implantation and auto-radiographic methods DA-99 \$4,850	APPROVED \$4,000

Donohue, W.B. Oral Pathology Montreal	Glycogen content and distribution in young and aged buccal mucosa DA-100 \$1,400	APPROVED \$1000
Dowse, C.M. & Kleinberg, I. Oral Biology Manitoba	Packard liquid scintillation and flow monitor system DE-13 \$11,610	APPROVED \$6,200
Dowse, C.M. Oral Biology Manitoba	Metabolism of Periosteum DA-81 \$13,870	APPROVED \$12,000
Edmund, A.G. Royal Ontario Museum Toronto	Dynamics of tooth replacement processes in modern reptiles DA-104 \$3,900	APPROVED \$3,700
Ellis, R.G., Dean Faculty of Dentistry, Toronto	Support of two undergraduate summer assistants DA-113 \$2,000	APPROVED \$2,000
Francis, L.E. Oral Pathology McGill	Further studies in tooth transplantation DA-88 \$2,000	APPROVED \$2,000
Francis, L.E. Pharmacology & Therapeutics McGill	Investigation of a gingival histamine and serotonin antagonist DT-76 \$5,000	APPROVED \$5,000 as a three-year Term Grant
Hunt, A.M. Dentistry Toronto	Measurement of strontium ⁹⁰ and carbon ¹⁴ in primary teeth of Canadian children DA-82 \$8,700	APPROVED \$8,000 as a three-year Term Grant
Jarvis, A.A. School of Hygiene Toronto	Cesium metabolism in bone \$6,000	NOT APPROVED
Jordon, R.E. Operative Dentistry Dalhousie	Primary molar development in the human fetus DA-106 \$2,000	APPROVED \$1,500

Kasloff, Z. Restorative Dentistry Manitoba	The relationship of defects in teeth associated with induced stresses to the histological pattern of tooth structure DA-90	APPROVED \$4,000 \$4,790
Kasloff, Z. Restorative Dentistry Manitoba	Metallographic studies of soldered joints and cast gold parent metals DA-108	APPROVED \$500 \$1,500
Khairat, O. Bacteriology & Immunology Manitoba	Post-extraction bacteraemia DA-101	APPROVED \$3,800 \$4,192.80
Kleinberg, I. Oral Biology Manitoba	1. Further studies on the role of the dental plaque in caries process 2. Further studies on the effect of substances removed during the refining of carbo- hydrates on calcium phosphate solubility DT-78	APPROVED \$4,000 Supplement to DT-78 \$5,000
Leblond, C. P. Anatomy McGill	Formation of matrix of enamel and dentin DT-23	APPROVED \$2,400 Supplement to DT-23 \$2,415
Leung, S. Wah Dentistry Br. Columbia	Basic and clinical research in dentistry	WITHDRAWN \$10,300
Lussier, J. P. Physiology Montreal	The origin and fate of citrate in bone under various influences DA-70	APPROVED \$5,800 WITHDRAWN \$6,780
Lussier, J. P. Dean, Faculty of Dentistry, Montreal	Support for two undergraduate summer assistants DA-116	APPROVED \$2,000 \$2,000

MacLean, H.R.	Support for two undergraduate	APPROVED \$2,000
Dean, Faculty of	summer assistants	
Dentistry,	DA-111	\$2,000
Alberta		
McCutcheon, J.	Support for two undergraduate	APPROVED \$2,000
Dean, Faculty of	summer assistants	
Dentistry,	DA-115	\$2,000
McGill		
McLean, J.D.	Support for two undergraduate	APPROVED \$2,000
Dean, Faculty of	summer assistants	
Dentistry,	DA-112	\$2,000
Dalhousie		
McMurchy, K.A.	"Prior" tissue culture unit	APPROVED \$2,700
Oral Histology	DA-69	\$2,850
& Pathology		
Alberta		
Mezl, Z.	Experimental modifications	APPROVED \$1,600
Oral Pathology	of the permeability of the	
Montreal	superficial zone of enamel	
	caries	
	DA-79	\$1,800
Neilson, J.W.	Support for two undergraduate	APPROVED \$2,000
Dean, Faculty of	summer assistants	
Dentistry,	DA-114	\$2,000
Manitoba		
Nikiforuk, G.	Technicon amino acid	WITHDRAWN
Dentistry	analyzer	
Toronto		\$8,201.60
Perreault, J.G.	Changes in the pulp connective	APPROVED \$3,500
Pediatric	tissue following irritation	
Dentistry	DA-80	\$3,900
Montreal		

Pritchard, E. T.	Effect of molybdenum on	APPROVED \$7,400
Oral Biology	sulfolipids in rat tissue	
Manitoba	DA-109	\$9,140
Pritchard, E. T.	Liquid chromatography	APPROVED \$4,000
Oral Biology	analytical system	
Manitoba	DE-14	
Rollo, I. M.	The role of anaerobic strepto-	NOT APPROVED
Pharmacology &	cocci found in the blood of	
Therapeutics	patients after tooth extraction	
Manitoba	in the aetiology of bacterial	
	endocarditis	
		\$9,001
Saunders, R. L. de C. H.	X-ray microscopy of the	APPROVED \$2,600
Anatomy &	vascularisation of the human	
Histology	dental pulp at various ages	
Dalhousie	DA-105	\$2,789.40
Sinclair-Hall, A. H.	Polyvinyl-alcohol sponge and	APPROVED \$1,500
Anatomy	the healing of bony defects	
Manitoba	DA-92	\$1,600
Spouge, J. D.	Hypersensitivity reactions	APPROVED \$2,000
Oral Biology	in the mucous membranes	
Manitoba	DA-93	\$2,985
Tuba, J.	A. Influence of dietary	APPROVED \$6,500
Dentistry	phosphate on dental caries	
Alberta	in the rat	
	B. Glycoproteins in gingival	
	tissue of the rat	
	DA-94	\$7,580
Woodside, D. G.	The relation between	APPROVED \$3,000
Orthodontics	mandibular growth rate and	
Toronto	form	
	DA-110	\$3,500
Youdelis, W. V.	Fundamentals of amalgam	APPROVED \$5,000
Mining and	metallurgy and development	
Metallurgy	of dental amalgams	
Alberta	DA-95	\$6,475

In summary a total of thirty-three new applications amounting to \$184,286 were considered. Of these thirty-three applications, two were for supplements to existing term grants, and three were for major equipment grants. Twenty-nine grants amounting to \$117,000 were recommended. Of these, two grants were for supplements and two grants were for major equipment. One major equipment application and one general grant application were withdrawn. A further \$12,000 was awarded to six Dental Faculties (\$2,000 each) for the support of two Special Summer Assistantships. These grants were made to the Deans of each Faculty.

It was MOVED by Dr. G. Nikiforuk and SECONDED by Dr. I. Kleinberg

THAT grants be made in the amounts specified.

CARRIED

During the discussion of the applications for those grants which were requested by attending members of the Committee, each committee member left the room until his case had been discussed and the recommendation had been made.

In view of Dr. G. Nikiforuk's withdrawal of his major equipment application

It was MOVED by Dr. A.J. Rhodes and SECONDED by Dr. A.G. Gornall

THAT his major equipment application be given special consideration at the 1964 annual meeting.

CARRIED

16. Allotment for 1963-64

The Committee allotment for 1963-64 was recommended for allocation as follows:

1 Associateship	\$ 9,600*
8 Fellowships	34,500
8 Term Grants	51,000
29 Annual Grants	116,000
Administration	2,000
Total	\$213,100

* Plus applicant's share of cost of University pension plan, etc.

17. Forms and Instructions

The Committee considered at length the problem of properly worded forms of all kinds, both applications and others, and decided that the forms and informational material distributed by the Committee should be reviewed with a view to providing a clearer picture of the work of the Committee and encouraging better worded and explained grant applications.

It was MOVED by Dr. A.G. Gornall and SECONDED by Dr. I. Kleinberg

THAT the Chairman be empowered to appoint a sub-committee which would examine the mode of applications, including forms, and recommend changes.

CARRIED

18. Membership of Committee

The Chairman informed the Committee that three new members should be elected to replace himself (Dr. K.J. Paynter), Dr. A.G. Gornall and Dr. G.D. Scott who were retiring. He also informed them that Dr. C.P. Leblond, Dr. G. Nikiforuk, and Dr. A.J. Rhodes had completed their first three-year terms and were eligible for reappointment for second three-year terms.

The following new members were duly nominated and elected: Dr. R.V. Blackmore of the University of Alberta, Dr. A.M. Hunt of the University of Toronto, and Dr. S. Wah Leung of the University of British Columbia.

It was MOVED by Dr. J.P. Lussier and SECONDED by Dr. I. Kleinberg

THAT the three new nominees and the three members eligible for reappointment be appointed for three-year terms terminating 31 March 1966.

CARRIED

It was MOVED by Dr. G. Nikiforuk and SECONDED by Dr. I. Kleinberg

THAT Dr. J.P. Lussier be appointed Chairman of the Committee for one year commencing 1 April 1963.

CARRIED

It was MOVED by Dr. C. Castaldi and SECONDED by Dr. I. Kleinberg

THAT the Chairman be given authority to appoint two replacements to the Executive Committee.

CARRIED

19. Estimates for 1964-65

It was decided that since there appeared to be great likelihood of increasing numbers of applications for research grants and scholarship support, the Committee should submit a request for \$292,000 to carry out its obligations in 1964-65.

20. Date of Next Meeting

Unless the Chairman felt it necessary to call a meeting to consider further applications, the next meeting of the Committee would be held at approximately the same time next year.

The meeting adjourned at 12:40 p.m. on 1 March 1963.

INDEX OF PROGRESS REPORTS 1962-63

<u>Grantee</u>	<u>Project</u>	<u>Appendix</u>
Anderson, D.L.	DA-86	A
Dale, J.G.	DA-99	B
Donohue, W.B. & Perreault, J.G.	DA-80	C
Donohue, W.B.	DA-100	D
Dowse, C.M.	DA-81	E
Francis, L.E.	DT-76	F
Francis, L.E.	DA-88	G
Hunt, A.M.	DA-82	H
Khairat, O.	DA-101	I
Kasloff, Z.	DA-90	J
Leblond, C.P.	DA-23	K
Leblond, C.P.	DA-23	L
Lussier, J.P.	DA-70	M
Mezl, Z.	DA-79	N
Saunders, R.L. de C.H. & Rockert, H.	DA-105	O
Sinclair-Hall, A.H.	DA-92	P
Tuba, J.	DA-94	Q
Youdelis, W.V.	DA-95	R

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH
NATIONAL RESEARCH COUNCIL

DECEMBER - 1962

NAME: DR. D. L. ANDERSON
INSTITUTION: FACULTY OF DENTISTRY, UNIVERSITY OF TORONTO
PROJECT TITLE: DA-86, "HISTOCHEMICAL INVESTIGATION OF CELL CYTOPLASM AND INTERCELLULAR INTER-FIBRILLAR MATERIAL."
CONTENTS: CONNECTIVE TISSUE REACTIONS:- GRANULOMA FORMATION

A. EFFECT OF AZURE A

1. AZURE A AND CARRAGEENAN
2. AZURE A TOXICITY

B. EFFECT OF DILANTIN

1. IN DRINKING WATER
2. INTRAMUSCULAR INJECTIONS
3. SUBCUTANEOUS INJECTIONS

C. EFFECT OF VARIATIONS IN CARRAGEENAN TECHNIQUE

1. CONCENTRATION
2. VOLUME
3. SITE

A. EFFECT OF AZURE A - 1. AZURE A AND CARRAGEENAN

INTRODUCTION

SULPHATED POLYSACCHARIDES ARE BELIEVED TO BE INTIMATELY ASSOCIATED WITH THE FORMATION OF COLLAGEN FIBERS, BUT THE

NATURE OF THIS RELATIONSHIP IS STILL OBSCURE. JUST PRIOR TO FIBER FORMATION, THE CONCENTRATION OF SULPHATED POLYSACCHARIDES BECOMES HIGH, AND THEN DECREASES AS THE FIBERS ARE FORMED. HIGH DOSES OF CORTISONE SUPPRESS BOTH SULPHATION OF POLYSACCHARIDES AND FIBER FORMATION. LATTES ET AL 1954, 1956, GAVE EVIDENCE TO SUGGEST THAT ACID MUCOPOLYSACCHARIDES COULD OVERCOME THE INHIBITORY EFFECT OF CORTISONE ON FIBER FORMATION.

THE SULPHATED POLYSACCHARIDE "CARRAGEENAN" CAN BE USED TO EVOKE FIBER FORMATION. I FOUND (1961) THAT IN MALE RATS CORTISONE DID NOT INHIBIT COLLAGEN FIBER FORMATION IN THE CARRAGEENAN GRANULOMA, ALTHOUGH IT COMPLETELY INHIBITED FORMATION OF THESE FIBERS IN SKIN WOUNDS AND IN IMPLANTED COTTON PELLETS IN THE SAME ANIMALS AT THE SAME TIME.

WILLIAMS, 1957, BENITZ AND HALL, 1959, AND FISHER AND PAAR, 1960, POSTULATED THAT THE HIGH DEGREE OF SULPHATION OF CARRAGEENAN IS PROBABLY AN IMPORTANT FACTOR IN EVOKING THE GRANULOMATOUS REACTION, FOR METHYL CELLULOSE, POLYVINYL ALCOHOL, DEXTRAN, PECTIC ACID, AND AGAR FAIL TO GIVE RESULTS SIMILAR TO CARRAGEENAN. (WILLIAMS, 1957).

METHOD

TO STUDY THE IMPORTANCE OF THE SULPHATE GROUPS OF CARRAGEENAN IN EVOKING FIBER FORMATION, A CATIONIC DYE, AZURE A, WAS COMBINED WITH CARRAGEENAN TO BLOCK THE SULPHATE GROUPS. SEVERAL METHODS WERE TRIED TO BLOCK THE SULPHATE GROUPS. SOME AGENTS SUCH AS PROTAMINE SULPHATE EFFECTIVELY BLOCKED THE SULPHATE GROUPS, BUT IN SO DOING PRECIPITATED THE CARRAGEENAN. IT WAS FOUND THAT 0.01% AZURE A COMBINED WITH 2% CARRAGEENAN

WITHOUT PRECIPITATION TO GIVE METACHROMASIA, - WHICH IS EVIDENCE TO UNION. HIGHER CONCENTRATIONS OF AZURE A PRODUCED PRECIPITATION, WHEREAS LOWER CONCENTRATIONS DID NOT GIVE THE METACHROMATIC REACTION.

GRANULOMAS DUE TO CARRAGEENAN WERE COMPARED TO THOSE PRODUCED BY THE COMBINED CARRAGEENAN-AZURE A IN THE SAME GUINEA PIGS. ONE CC. OF 1% CARRAGEENAN IN NORMAL SALINE WAS INJECTED SUBCUTANEOUSLY IN THE LOWER RIGHT ABDOMINAL REGION. ONE CC. OF 0.005% AZURE A IN 1% CARRAGEENAN IN NORMAL SALINE WAS INJECTED SUBCUTANEOUSLY IN THE LOWER LEFT ABDOMINAL REGION. SOME ANIMALS ALSO HAD ONE CC. OF 1% AGAR IN NORMAL SALINE INJECTED SUBCUTANEOUSLY IN THE UPPER RIGHT ABDOMINAL REGION. PENICILLIN WAS ALSO GIVEN TO THE ANIMALS AT THE TIME OF IMPLANTATION. AFTER TWO WEEKS, THE GRANULOMATA WERE REMOVED AND WEIGHED. SOME WERE DRIED IN AN OVEN AT 60⁰ C. AND REWEIGHED AT ONE AND TWO WEEKS. THOSE ANIMALS IN WHICH ALL GRANULOMATA WERE NOT DISTINCTLY FORMED WERE OMITTED, AS A COMPARISON BY WEIGHT THUS COULD NOT BE MADE. GUINEA PIGS WERE USED RATHER THAN RATS BECAUSE OF THE GREATER GRANULOMATOUS REACTION TO CARRAGEENAN IN THE GUINEA PIG. THIS IS IMPORTANT IN RESPECT TO THE SMALL VOLUMES INJECTED.

RESULTS

CARRAGEENAN AND CARRAGEENAN-AZURE A GRANULOMATA

AREA, IN SQ. MMS.

CARRAGEENAN	CARRAGEENAN-AZURE A	%CARR.-AZURE A IS OF CARR.
440	234	53.2

CARRAGEENAN	CARRAGEENAN-AZURE A	%CARR-AZURE A IS OF CARR.
644	315	49.0
405	210	51.8
400	104	26.0
375	250	66.7
425	272	64.0
375	195	52.0
288	60	20.8
MEANS 419	205	48.0

WEIGHTS, IN MGS.

WET			DRY		
CARRAGEENAN	CARRAGEENAN-AA	%	CARRAGEENAN	CARR-AA	%
438	184	42.0	133	68	51.1
735	275	37.4	190	74	38.9
382	199	52.2	82	46	56.1
562	253	45.0	110	60	54.5
137	44	32.1	31	12	38.7
MEANS 451	191	41.7	109	52	47.5

THERE IS A HIGHLY SIGNIFICANT DIFFERENCE BETWEEN THE CARRAGEENAN AND CARRAGEENAN-AZURE A GRANULOMATA WEIGHTS,

$P < .01$.

CONCLUSIONS.

THUS, IT WAS FOUND THAT BLOCKADE OF THE SULPHATE GROUPS REDUCED THE WEIGHT AND SIZE OF THE GRANULOMATA TO LESS THAN HALF THAT OF THE UNBLOCKED CARRAGEENAN, AND HENCE INDICATES THAT THE SULPHATE GROUPS PLAY AN IMPORTANT ROLE IN THE FIBER

FORMATION.

CARRAGEENAN, TYPE 21, WAS KINDLY SUPPLIED BY MR. M. L. STOLOFF, SEAPLANT CORPORATION, BEDFORD, MASS.

A. EFFECT OF AZURE A - 2. AZURE A TOXICITY.

INTRODUCTION

THIS INVESTIGATION WAS DONE TO CHECK IF THE REDUCTION OF THE CARRAGEENAN GRANULOMA DUE TO AZURE A WAS A RESULT OF A TOXIC EFFECT OF AZURE A ON THE CELLS.

AZURE A IS REPORTED TO BE NON TOXIC. IT IS USED CLINICALLY IN HUMANS IN "DIAGNEX BLUE." SQUIBB'S 1958 BOOKLET STATES THAT NO TOXIC EFFECTS HAVE BEEN OBSERVED IN THE THOUSANDS OF GASTRIC ANALYSES PERFORMED WITH DIAGNEX BLUE. HCl OF THE STOMACH RELEASES THE AZURE A FROM THE RESIN AND THE DYE ENTERS THE BLOOD STREAM AND IS ELIMINATED IN THE URINE WHERE IT IS MEASURED. WHEN LARGE DOSES WERE ADMINISTERED TO DOGS AND TO RATS, NO SIGNIFICANT CHANGES IN ANY RESPECT WERE NOTED.

METHOD

TO TEST THE EFFECT ON GUINEA PIGS, 5 MG. OF AZURE A (1 CC. OF 0.5% AZURE A IN 0.5% PROTAMINE SULPHATE), WAS INJECTED INTRAPERITONEALLY DAILY FOR 14 DAYS IN THREE GUINEA PIGS. PROTAMINE SULPHATE WAS USED TO OVERCOME THE EFFECT OF THE HEPARIN IN THE BLOOD, SO THAT THE DYE MIGHT REACH THE EXTRAVASCULAR CONNECTIVE TISSUE. (CSABA ET AL, 1960).

RESULTS

IT WAS NOT EVIDENT THAT THE DYE DID REACH THE EXTRA-VASCULAR CONNECTIVE TISSUE, FOR IT WAS NOT NOTED IN 8 MM. DIAMETER WOUNDS MADE IN THE SKIN OF THE BACK OF THE ANIMALS. THE DYE, HOWEVER, WAS EVIDENT IN THE URINE AND TEARS. ON EXAMINATION OF THE ANIMALS THERE WERE NOT ILL EFFECTS EVIDENT.

CONCLUSIONS

THERE IS NO EVIDENCE THAT AZURE A IS TOXIC. EACH DAILY DOSE OF AZURE A WAS 100 TIMES THAT CONTAINED IN THE MIXTURE USED IN THE SINGLE INJECTION WITH CARRAGEENAN. IN THIS LATTER INSTANCE, THE AZURE A ALSO WAS BOUND TO THE CARRAGEENAN AND IS NOT APT TO BE RELEASED IN THE TISSUES FOR CARRAGEENAN IS A VERY STRONG CHROMOTROPE. THUS, IT IS VERY UNLIKELY THAT THE EFFECT OF AZURE A IN REDUCING THE SIZE OF THE CARRAGEENAN GRANULOMA WAS DUE TO TOXICITY OF AZURE A.

B. EFFECT OF DILANTIN - I. IN DRINKING WATERINTRODUCTION

DILANTIN ADMINISTRATION FREQUENTLY RESULTS IN THE ENLARGEMENT OF HUMAN GINGIVA. THE ENLARGEMENT IS DUE CHIEFLY TO THE PRODUCTION OF COLLAGEN FIBERS. THE MECHANISM IS STILL OBSCURE, AND ITS INVESTIGATION HAS BEEN HAMPERED BY THE DIFFICULTY MANY HAVE EXPERIENCED IN OBTAINING GINGIVAL ENLARGEMENT IN EXPERIMENTAL ANIMALS GIVEN DILANTIN.

EVIDENCE SUGGESTS THAT DILANTIN EXAGGERATES RESPONSES IN WHICH COLLAGEN PRODUCTION IS ALREADY INITIATED.

ACCELERATED WOUND HEALING IN HUMAN GINGIVA WAS REPORTED BY SHAPIRO, 1958. INCREASED TENSILE STRENGTH OF HEALING WOUNDS IN RAT SKIN WAS REPORTED BY KELLN AND GORLIN, 1960.

IN THE FOLLOWING EXPERIMENT, CARRAGEENAN AND COTTON PELLETS ARE USED TO INITIATE FIBER PRODUCTION. THE FIBERS ARE FORMED AS PART OF THE RESPONSE TO LOCAL IRRITATION. THE ROLE LOCAL IRRITATION PLAYS IN DILANTIN-INFLUENCED GINGIVAL ENLARGEMENT IS NOT CLEAR, BUT A RECENT REPORT BY PANUSKA ET AL, 1961, SUGGESTS THAT A RELATIONSHIP DOES EXIST.

THERE ARE ADVANTAGES IN USING THESE IMPLANTS OVER USING WOUNDS TO STUDY FIBER PRODUCTION. AN ENLARGEMENT IS PRODUCED, AND THIS IS CLOSER TO THE CLINICAL GINGIVAL PROBLEM. THERE IS MORE TISSUE TO WORK WITH, IT CAN BE MORE READILY DIFFERENTIATED FROM PRE-EXISTING TISSUE, AND IT CAN BE WEIGHED. ALSO, THE PRODUCTION OCCURS OVER A LONGER PERIOD OF TIME, AND THIS SHOULD BE MORE PERTINENT IN REGARD TO DILANTIN ADMINISTRATION, AS IT IS USUALLY PROLONGED.

METHOD

FOUR MONTH OLD ALBINO RATS WERE USED. SEVEN FEMALES AND SIX MALES RECEIVED DILANTIN. THREE OF EACH SEX WERE CONTROLS. DILANTIN WAS GIVEN ORALLY 8 TIMES IN THE DRINKING WATER, IN A CONCENTRATION OF 0.0658 MG. PER CC. THIS WAS GIVEN 3 DAYS BEFORE IMPLANTATIONS, AND THEREAFTER UNTIL SACRIFICE. FLUID INTAKE OF EACH ANIMAL WAS MEASURED DURING THE 17 DAYS.

IMPLANTATIONS WERE MADE SUBCUTANEOUSLY. Two no.3 DENTAL COTTON PELLETS WEIGHING BETWEEN 4 1/4 AND 5 3/4 MGS. WERE IMPLANTED IN THE BACK THROUGH A MID LINE INCISION. FIVE CC. OF 1% TYPE 21 CARRAGEENAN IN NORMAL SALINE WAS INJECTED IN THE LOWER ABDOMINAL REGION. ON SACRIFICE, PELLETS AND GRANULOMATA WITH ATTACHED SKIN WERE REMOVED AND WEIGHED.

RESULTS

THE DOSE OF DILANTIN RECEIVED IN THE DRINKING WATER VARIED BETWEEN 9 AND 20 MG./KG./DAY. AVERAGE FLUID INTAKE WAS 65 CC. FOR 368 GM. MALES, AND 50 CC. FOR THE 214 GM. FEMALES. THUS, THE AVERAGE DAILY DOSE WAS 12 MG./KG./DAY FOR THE MALES, AND 15 1/2 MG./KG./DAY FOR THE FEMALES. EVEN IF INJECTIONS HAD BEEN USED, 4 MG. IN RATS VARYING AS THESE DID BETWEEN 200 AND 407 GMS. WOULD HAVE MEANT A DOSE VARIATION OF 10 TO 20 MG./KG./DAY. THERE WAS NO INDICATION THAT ANY DRINKING BOTTLES EMPTIED ON THEIR OWN, AND ALTHOUGH THERE WAS PROBABLY A LITTLE LEAKAGE IN ALL, THE DOSE STILL WAS GREATER THAN 8 MG./KG./DAY, WHICH IS THE MAXIMUM THERAPEUTIC DOSE FOR HUMANS. WITH 7 MG./KG./DAY ISHIKAWA AND GLICKMAN, 1961, OBTAINED GINGIVAL ENLARGEMENT IN CATS.

MEAN WEIGHTS OF GRANULOMATA IN MGS:-

COTTON PELLETS:

26 IN DILANTIN TREATED ANIMALS WEIGHED 55 MG. WET AND 12.8 MG. DRY.

12 IN CONTROL ANIMALS WEIGHED 50 MG. WET AND 13.4 MG. DRY.

SKIN-CARRAGEENAN GRANULOMATA:

	MALE	FEMALE
DILANTIN TREATED	3884	2654
CONTROL	3003	2364

THERE IS NO SIGNIFICANT DIFFERENCE BETWEEN DILANTIN TREATED AND CONTROL (F IS 2.7).

CONCLUSIONS

DILANTIN AS USED HAD NO EFFECT ON EITHER CARRAGEENAN OR COTTON PELLET GRANULOMATA. IN A DISCUSSION OF A PAPER (ANDERSON, 1962) IT WAS SUGGESTED THAT IN RATS A HIGHER DOSE OF DILANTIN IS NECESSARY.

B. EFFECT OF DILANTIN - 2. INTRAMUSCULAR INJECTIONSINTRODUCTION

A PILOT EXPERIMENT WAS DONE TO SEE IF THE TISSUE FORMATION IN CARRAGEENAN AND COTTON PELLET GRANULOMATA COULD BE INCREASED BY USING HIGH DOSES OF DILANTIN PLUS DURABOLIN. DURABOLIN (ORGANON INC.) IS REPORTED TO INCREASE ANABOLISM. IT IS MORE POTENT THAN TESTOSTERONE PROPIONATE IN THIS REGARD BUT HAS LESS EFFECT ON SECONDARY SEX CHARACTERISTICS (OVERBEEK AND DE VISSER, 1957, KIM, 1961).

METHOD

TWO GUINEA PIGS AND TWO RATS, ALL MALE, WERE USED. INTRAMUSCULAR INJECTIONS OF DILANTIN AND DURABOLIN WERE GIVEN. 5 INJECTIONS OF 0.3 CC. OF 10% DILANTIN, AND 3 INJECTIONS OF 0.05 CC. OF 2.5% DURABOLIN IN SESAME OIL WERE MADE, 4 DAYS BEFORE IMPLANTATION AND AT SPACED INTERVALS DURING THE TWO WEEK IMPLANTATION PERIOD.

5 CC. OF 1% CARRAGEENAN IN NORMAL SALINE WAS IMPLANTED SUBCUTANEOUSLY IN THE LOWER ABDOMINAL AREA, AND TWO 4 1/4 TO 5 3/4 MG. COTTON PELLETS WERE IMPLANTED SUBCUTANEOUSLY IN THE BACK.

RESULTS

ONE RAT DIED. IN THE GUINEA PIGS THE COTTON PELLET WEIGHT WAS 66 MG. AND CARRAGEENAN GRANULOMATA AVERAGED 3950 MG. IN THE SURVIVING RAT, THE PELLETS WEIGHED 62 MGS. AND THE CARRAGEENAN GRANULOMA WEIGHED 1506 MG. THESE WEIGHTS ARE NO GREATER THAN THOSE OBTAINED IN CONTROL ANIMALS IN IMPLANTATIONS IN OTHER STUDIES.

CONCLUSION

DILANTIN AND DURABOLIN AS USED HAD NO APPARENT EFFECT ON THE GRANULOMATA WEIGHTS.

B. EFFECT OF DILANTIN - 3. SUBCUTANEOUS INJECTIONS

INTRODUCTION

WHEN IT WAS FOUND THAT CARRAGEENAN GRANULOMATA COULD BE MORE ACCURATELY COMPARED IN THE SAME ANIMAL THAN IN DIFFERENT ANIMALS, (SEE PART C3 OF THIS REPORT), IT WAS DECIDED TO TRY TO USE DILANTIN LOCALLY WITH CARRAGEENAN.

METHOD

EIGHT WHITE RATS WERE USED. INJECTIONS WERE MADE SUBCUTANEOUSLY IN THE RIGHT AND LEFT LOWER ABDOMINAL AREA, AND LEFT FOR TWO WEEKS. 1 CC. OF 1% CARRAGEENAN IN NORMAL SALINE WAS IMPLANTED ON THE RIGHT SIDE. 1 CC OF 1% CARRAGEENAN IN 10% DILANTIN WAS IMPLANTED ON THE LEFT SIDE.

RESULTS

ALL DILANTIN-CARRAGEENAN COMBINATION SITES ABSCESSED

AND THEN PERFORATED THE SKIN. AT TWO WEEKS, THERE WAS STILL WHITE GRANULAR SPONGY MATERIAL IN THE SKIN AT THE INJECTION SITE.

CONCLUSION

THE IRRITATION PRODUCED WAS TOO STRONG FOR THE INTENDED PURPOSE OF THE EXPERIMENT.

C. EFFECT OF VARIATIONS IN CARRAGEENAN TECHNIQUE

INTRODUCTION

THE GRANULOMATOUS REACTION TO CARRAGEENAN IN REGARD TO VARIATIONS IN CONCENTRATION, VOLUME, AND SITE OF INJECTION WAS INVESTIGATED. THIS WAS DONE TO LEARN UNDER WHAT CONDITIONS CARRAGEENAN CAN BEST BE USED TO ASSESS CONNECTIVE TISSUE CAPABILITIES. IT HAS BEEN STATED (ABERCROMBIE ET AL, 1961) THAT THE FORMATION AND REMOVAL OF COLLAGEN IN THE GRANULOMATOUS REACTION TO CARRAGEENAN IS SIMILAR TO THAT WHICH OCCURS IN GRANULATION TISSUE HEALING WOUNDS.

METHOD, RESULTS, AND CONCLUSIONS

1. CONCENTRATION

ONE, TWO AND THREE PER CENT. SOLUTIONS WERE INJECTED IN THE SAME GUINEA PIGS. FOUR ANIMALS WERE USED, AND 0.5 CC. VOLUME WAS USED. THE GRANULOMATA WERE ASSESSED AT 14 DAYS. IT WAS FOUND THAT THE GREATER THE CONCENTRATION, THE GREATER WAS THE REACTION.

2. VOLUME

ON COMPARING 0.5 CC, 1.0 CC., AND 5.0 CC. INJECTIONS, AT 1% CONCENTRATION IN DIFFERENT GUINEA PIGS, IT WAS FOUND THAT THE GREATER THE VOLUME, THE GREATER WAS THE REACTION. WITH INCREASE IN VOLUME THERE WAS, HOWEVER, ALSO AN INCREASE IN QUANTITY OF CARRAGEENAN.

3. SITE

A. SAME ANIMAL

ON COMPARING THE THORAX AND LOWER ABDOMEN AS INJECTION SITES, IT WAS FOUND THAT WITH EQUAL VOLUMES AND CONCENTRATION, A GREATER REACTION OCCURRED IN THE LOWER ABDOMINAL REGION.

B. DIFFERENT ANIMALS

ON COMPARING CONSTANCY OF REACTION TO CARRAGEENAN, IT WAS FOUND THAT WITH EQUAL VOLUMES AND CONCENTRATIONS, THERE WAS CONSIDERABLY MORE VARIATION BETWEEN DIFFERENT GUINEA PIGS THAN BETWEEN TWO LOWER ABDOMINAL SITES IN THE SAME ANIMAL. THE WEIGHTS AND AREAS IN THE TABLES COMPARING THE AZURE A-CARRAGEENAN AND CARRAGEENAN GRANULOMATA ARE GOOD EXAMPLES OF THIS (SEE PART A1 OF THIS REPORT).

OTHER EXAMPLES:

0.5 cc. 3% - ANIMAL A 3974 AND 3153 MGS.

B 1231 AND 1038 MGS.

1.0 cc. 1% - ANIMAL C 35 AND 35 SQ.MM.

D 60 AND 70 SQ.MM.

E 600 AND 900 SQ.MM.

THESE RESULTS INDICATE THAT CARRAGEENAN INJECTION IS A TECHNIQUE THAT CAN BE USED TO ASSESS CONNECTIVE TISSUE, AND WILL SHOW DIFFERENCES IN CONNECTIVE TISSUE CAPABILITIES IN DIFFERENT ANIMALS.

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REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH
NATIONAL RESEARCH COUNCIL

December, 1962

Name: Jack G. Dale
Institution: Faculty of Dentistry, University of Toronto
Project: DA-99. The Study of Bone Resorption by
Implantation Methods.

Included in the application for a grant in aid of research, operating grant DA-99, 1962-63, were five "specific aims" of proposed investigation:

1. to determine whether bone resorption can occur without the presence of osteoclasts and other cells,
2. to study various local conditions under which multi-nucleated cells appear in the tissues, and to determine whether resorption is taking place,
3. to study the local and systemic influence of hormones and nutrients on bone resorption,
4. to observe the resorptive process in the living animal with and without the presence of cells,
5. to study the ultra structure of resorbing bone and multi-nucleated cells.

The procedures involved in 1, 2, and 3 are similar in many respects, and involve the use of three radioactive isotopes: Ca^{45} to represent the inorganic component of bone implants, S^{35} to represent the ground substance fraction of the organic component, and tritiated glycine to represent the collagen fraction of the organic component. Since this is the first series of investigations utilizing these specific procedures, it was decided that a programme of preliminary

experiments should be carried out to determine the specific dosage level, the method of administration of the isotopes, and the preparation of the sections.

Pilot studies have been completed and results have been obtained for Ca^{45} and S^{35} . Major experiments utilizing Ca^{45} have been completed and are being processed.

Method of Procedure

A large portion of the right scapula was removed from young rats of the Holtzman albino strain, under rigid sterile conditions and ether anesthesia. Immediately upon removal, the bone was placed into a sterile test tube and devitalized by alternately freezing with dry ice for 30 minutes, and thawing in warm sterile saline for 30 minutes. This procedure was repeated three times in order to kill all associated cells. The scapula was then divided into 4 equal portions. One was prepared immediately for use as a reference, to give the histological and radioautographical picture, as well as the specific activity of the bone before implantation. The second was placed directly into the subcutaneous tissue of the right side of a litter-mate's back for use as a control. The third was placed in a physiological saline solution containing transudate levels of calcium and phosphorous for use as a second control and the fourth was sealed within a diffusion chamber to separate it from host cells, and was placed in the subcutaneous tissue of the right side of a second litter-mate's back. The latter serves as an experimental implant. Each diffusion chamber consists of two sterile cellulose ester

Millipore filters, 19 mm. in diameter, 150μ in thickness, containing pores $.45\mu$ in diameter, reinforced with nylon microweb, and cemented together with Millipore filter cement which consists of cellulose ester dissolved in acetone. Specially designed instruments were used in the cementing of the chamber. Ninety-six rats, weighing 80-100 grams, were used in the study, in a manner described in Table #1, Subgroup No. 1.

TABLE #1

EXPERIMENTAL DESIGN

DIVISION A 144 rats					DIVISION B 144 rats				
Radioactive donor, normal recipient					Normal donor, radioactive recipient				
Subgroup No. 1 Ca ⁴⁵ (half life 152 days)					Subgroup No. 1 Ca ⁴⁵				
Donor Animal	Recipient Animals		Number Animals	Duration of Implantation	Same				
	Cont. Expt.								
1a	1b	1c	3	1 week					
2a	2b	2c	3	2 weeks					
3a	3b	3c	3	3 weeks					
4a	4b	4c	3	4 weeks					
5a	5b	5c	3	8 weeks					
6a	6b	6c	3	12 weeks					
7a	7b	7c	3	16 weeks					
8a	8b	8c	3	20 weeks					
8	16	16	24	24	Total Animals		Total Animals 48		
				48					
Subgroup No. 1 Repeated			24						
Subgroup No. 2 S ³⁵ (half life 87 days) SAME					Subgroup No. 2 S ³⁵ SAME				
Subgroup No. 3 Cl ¹⁴ (long half life) SAME or Tritiated glycine					Subgroup No. 3 Cl ¹⁴ SAME				
TOTAL ANIMALS					144				
					144				

Donor animals provided 4 pieces of bone.

1. One for control animal (see Table)
2. One for experimental animal (see Table)
3. One for processing immediately - reference
4. One for physiological saline solution - second control.

"Hot bone" was placed into "cold transudate" to control "wash-out" and exchange (Division A of Table).

"Cold bone" was placed into "hot transudate" for cross check (Division B of Table).

This second control was used in an effort to account for absorption and exchange vs. mineralization and demineralization.

The dosage and dosage schedule to achieve desired in vivo labelling was determined by pilot experiments.

Ca^{45} was administered as a neutral ionic Ca salt and S^{35} as Na_2SO_4 .

Data to be obtained

1. Specific Activity of components of bone under consideration (Ca^{45} - inorganic, S^{35} and tritiated glycine).
2. Fecal and Urinary Elimination of Ca^{45} and S^{35} will be determined. It will be carried out in experiments involving radioactive donors, normal recipients.
3. Radioautographs will give indication of microdistribution of isotope, cellular localization and direction of diffusion in area of chamber.

4. Histochemical Procedures Hematoxylin and Eosin (to provide general histological structure); Periodic Acid Schiff, Van Giesen, Gomori Silver Nitrate (to identify the major components of bone); and Trypan Blue Injection (to differentiate osteoclasts from foreign body giant cells and to identify macrophages).

Use of Data

1. The specific activity will determine the relative contribution of exchange and non-specific adsorption processes vs. specific activity processes of solution and dissolution. If the specific activity becomes less in the "hot" experimental implant it would suggest that mineralization of the implant is occurring rather than demineralization.
2. If significant amounts of Ca^{45} and S^{35} are found in the fecal and urinary determinations, it would suggest that these ions are leaving the implant. This could, therefore, be interpreted as implant demineralization and/or degradation of the organic matrix.
3. Dissolution of the radioactive implant in the normal recipient might be indicated in the radioautographs by the presence of labelled components outside the chamber. If the "cold" implant becomes highly radioactive in the radioactive recipient, it might suggest deposition on the implant.
4. Histochemical procedures will be used to correlate with the radioautographs in this study and with the histochemical

procedures in the first investigation.

Major experiments utilizing S³⁵ are being prepared at the present time.

Preliminary investigations have been conducted utilizing the double chamber diffusion chamber and parathyroid gland tissue. These studies have been conducted to study the local influence of parathyroid hormone on bone resorption in a cell-free environment.

Time-lapse cinematography equipment has been purchased and preliminary investigations have been started. The purpose of this series of experiments is to observe the resorptive process in the living animal with and without the presence of cells.

Major experiments, designed to study cellular activity associated with bone resorption under the influence of an applied force, have been conducted utilizing tritium labelled thymidine.

An Autoradiographic Study of Cell Activity
During Tooth Movement

(Abstract to be presented at the International Association for Dental Research meeting, Pittsburg, 1963.)

Tritium labelled thymidine was used to study mitotic activity and the sequence of cell proliferation in the supporting structures of teeth under the influence of an applied force. Sixty-six female rats of the Wistar strain, 40 days of age, were divided into three equal groups. Each animal was given a single injection of H³ equivalent to 1 μ c/gm. of body weight. Force was applied by inserting rubber dam elastic between the right first and second maxillary molars. The left

molars served as a control. The experimental period extended from 4 hours to 21 days in all three groups. In group A, the animals were killed at varying intervals after injection and force application. In groups B and C, the injection was given at varying intervals after force application. Each animal was killed 2 hours after injection in group B, and 3 days after injection in group C. In tension areas, labelled cells were observed in the trans-septal fiber area, and adjacent to the cementum and alveolar bone surfaces. In pressure areas, labelled cells were not observed during the early periods after force application. Excessive pressure areas were characterized by contact of tooth and bone, root resorption and absence of cell proliferation. Labelled cells were observed at the periphery of pressure areas 4 hours after force application.

A series of supplementary experiments, designed to study cellular activity in the developing temporomandibular joint, have been conducted utilizing tritium labelled thymidine.

An Autoradiographic Study of the Developing
Temporomandibular Joint

(Abstract presented at the Second Canadian Conference on Dental Research, Banff, Alta., October, 1962.)

Thymidine has been shown to be an essential precursor of deoxyribonucleic acid (DNA). It has also been shown that it is not used in the synthesis of any other material in the body. Tritium-labelled thymidine, when injected into animals, becomes incorporated into the nuclei of those cells which are

synthesizing DNA during the period of chromosomal replication prior to dividing. Thus, the sequence of cell proliferation and migration may be followed in tissues by means of autoradiography.

This method was employed to study the cellular activity in the various structures of the developing temporomandibular joint. Twenty-one female rats of the Wistar strain were divided equally into three age groups: 2 days, 10 days, and 30 days. Each animal was given a single intraperitoneal injection of tritium-labelled thymidine equivalent to 1 microcurie per gram of body weight. One animal from each age group was killed 1/2 hour, 1 hour, 4 hours, 1 day, 2 days, 4 days, and 6 days after injection.

During the early periods following injection, labelled cells were observed throughout the joint except in the maturing zone of cartilage of the condyle. During the later periods, cells of the maturing zone of cartilage were also labelled. In the youngest animals, (1) cell proliferation was the most active, (2) the transitional zone, between the cartilage and the fibrous articular surface was the most extensive, and (3) the migration of labelled cells from the transitional zone to the maturing zone of cartilage was the most rapid. The reverse was true in the oldest animals. Facilities for the use of tritium labelled thymidine are well established in the research division, and it was decided to take advantage of this procedure if it could offer valuable information to our field of research.

In addition, during the initial year much time has been devoted to the necessary activities involved in transfer from Boston to Toronto, the establishment of facilities in the new location, the ordering of equipment and supplies, the training of personnel in the established procedures and the testing of new methods.

APPENDIX C

THE EFFECT OF A DOSE OF 2,000 r. OF X-RAY IRRADIATION ON THE GROWTH OF THE JAWS OF KITTENS.

By

William Beaugrand Donohue

and

J. Georges Perreault

Final Report on the work carried out with

the National Research Council grant D.A.80,

for the year 1959.

THE EFFECT OF A DOSE OF 2,000 r. OF X-RAY IRRADIATION
ON THE GROWTH OF THE JAWS OF KITTENS.

I Introduction.

II Review of the Literature.

III Material.

IV Methods.

A) Irradiation

1. Technique
2. Sedation
3. Weight

B) Injection of Alizarine Red S.

C) 1. Preparation of Ground Sections

2. Measurement of the Rate of Growth of Dentin in width.
3. Measurement of the Rate of Growth of Dentin in Length.

D) Decalcification, Sectioning and Staining.

E) Post Radiation Skull X-Rays.

1. Anatomic Points of Reference used in Measurements of the Lateral Skull X-Rays.

V Findings.

A) General Effects

1. Weight

B) Local Effects

1. Depilation
2. Ophthalmologic signs

C) Microscopic Findings: Teeth

1. Rate of growth of Dentin, as measured by Alizarine Red S.

1. Pattern of Dentin Growth.

2. Rate of growth in width.

3. Rate of growth in length.

11. Morphological Findings.

1. Enamel.

2. Dentin.

3. Pulp.

4. Periodontal Membrane.

D) Roentgenographic Findings: Teeth.

E) Rate of Eruption.

F) Microscopic Findings: Temporomandibular Joint.

G) Microscopic Findings: Sagittal Suture.

H) Appositional Growth of Bone Tissue of the Jaws.

I) Effect on the Growth of the Facial Skeleton.

VI Discussion.

A) Growth of Dentin in Length.

B) Growth of Dentin in Width.

C) Eruption of Teeth.

D) Growth of Bone Tissue.

E) Growth of the Facial Skeleton.

F) Lateral Resorption of the Incisor Teeth.

VII Summary and Conclusions.

VIII Bibliography.

IX Illustrations.

I. INTRODUCTION

The cat dentition is a heterodont and diphyodont type of dentition which somewhat resembles that found in the human. The deciduous dentition is completed when the animal is about 1.5 months old, and is replaced completely by the permanent dentition when the animal is about 5 months old. Thus, during this period the jaws present embryonic dental tissues demonstrating cell proliferation and differentiation, as well as formation of dentin, enamel and cementum. Furthermore, the jaws at this stage also present a dentition where maturation is complete. Thus, in the histologic sections of the jaws at this period, tooth development both during its inception and its development may be seen.

During this period, the growth centers of the jaws and skull are in an active stage of proliferation.

It is known that embryonic tissues are much more sensitive to x-ray irradiation than are more mature tissues (Bloom '48). It is also known that tissues of different embryonic origin have a different degree of susceptibility to x-ray irradiation (Desjardins '32; Warren '44).

The purpose of this study was to examine the effect of x-ray irradiation on the growth of the jaws in young cats; to study and compare the effects of such irradiation on the different tissues of the tooth during its development, as well as its effect on some of the other growth centers of the jaws.

II. REVIEW OF THE LITERATURE.

The effect of x-ray irradiation on the growth of the teeth.

Heretofore most of the studies on the effects of x-ray irradiation on the dental structures were limited to clinical observation (Regato '39; Dechaume, Cauhépe and Caudaert '49; Bruce and Stafne '50).

The majority of microscopic investigations have been limited to studies on the rodent incisor, frequently these were intended to corroborate the clinical findings of the author rather than present a thorough independent histologic investigation (Leist '27; Smith '38).

Tribondeau and Récamier ('05) first noted the effects of x-ray irradiation on the jaws, during a study which was primarily concerned with its effects on the eye. They did not report the dosage of x-ray radiation administered, but described an asymetry of the facial skeleton, and the fact that the teeth on the side of the radiation were smaller than on the control side.

Leist ('27) investigated histologically the effects of irradiation on the continuously growing rat incisor, and on a few dogs. He concluded that the odontoblasts were the most radiosensitive elements of the tooth. After irradiation they were fewer in number and completely disarranged, especially those of the coronal portion of the growing tooth.

Smith ('31) prompted by the earlier investigations of Leist, tried to determine whether the amount of radiation received by children during a full mouth roentenographic survey,

would effect the developing rat incisors. He could find no apparent changes, using the following irradiation method: S.T.D. 7 inches; 10 m.a.; a spark gap of 3 inches, and three to six exposures of from 5-20 seconds each with a dental x-ray machine. Later ('38) using higher doses he was able to reproduce some of Leist's findings.

Burstone ('50) in a series of experiments with 7 day and 28 day old mice, using a dose of 5000 r., and survival periods of one, two and three weeks, concluded that the degree of damage to the teeth and jaws was dependent on the age at which the mouse was irradiated, and the state of histogenesis of the individual tooth. In his one week survival group he noted distortion of both the ameloblasts and odontoblasts. However the odontoblasts were affected by lower doses of x-ray irradiation (1500 r. - 3000 r.) than were the ameloblasts. He observed also, that the more undifferentiated odontoblasts, exhibited a greater radiosensitivity than the mature odontoblasts. His experimental groups exhibited a metaplasia of the ameloblasts to a squamous and low cuboidal type of epithelium. Whereas the odontoblasts in some areas were noted to form an amorphous, eosinophilic type of matrix.

Burstone ('50 and '51) repeated his experiments using radioactive phosphorus, as a source of radiation in doses of from 15-60 microcuries per gram of body weight. Again he noted a cessation of histodifferentiation of ameloblasts, as well as metaplasia. The odontoblasts showed formation of osteodentin, and in animals that survived for more than two

weeks, there was a cessation of morphodifferentiation of the root structure. In the pulpal connective tissue he observed a form of hydropic degeneration.

Medak, Shour and Klauber ('50) observed the effects of x-ray irradiation in the 2000 r. to 2500 r. range on the rate of eruption of the rat incisor. They observed an initial increase in the rate of eruption in one group of animals, followed by a deceleration and finally complete cessation of eruption.

In the second part of this experiment the hypophysis was irradiated while shielding the jaws. They reported that no detectable changes could be seen in the teeth. The latter part of the experiment was repeated by English ('56) who confirmed that irradiation of the pituitary gland alone did not noticeably affect the dental system.

Bruce ('50) using radon needles as an external source of radiation reported that there was a retardation of the growth of the skull and the dental system in hamsters, if they were exposed during the first week following birth; however no measurements were made.

English and Tullis ('51) used young swine as their experimental animals; using a total radiation dose of 250 r. to 800 r., but with an extremely high kilovoltage of 2000 k.v. They noted massive haemorrhages in the crypts of the developing teeth and occasional haemorrhages in the pulp. They observed a hypoplasia of the enamel of the molars which they attributed to the sensitivity of the ameloblasts while actively producing matrix.

Medak, Weinreb, Sicher, Weinmann and Schour ('52) found that x-ray irradiation in the 1000 r. to 4000 r. range led to an anomaly of the outline of the tooth in the rat, which they described as a folding or a waviness of the enamel surface and dentino-enamel junction. This observation was explained by the difference in the growth rate between the pulp, dentin and enamel. Medak, Oartel and Burnett ('54) used the hamster, and administered x-ray irradiation in daily doses of 3000 r.; alternating the right and left sides of the head, until a total dose of 4800 r. had been delivered. They reported an initial oedema in the basal end of the upper incisors, which progressed by the fourth post-experimental week to a liquefaction necrosis, and finally to the formation of a cystic cavity. Unlike the rat, where the odontogenic epithelium resumes its activity following irradiation, there was no resumption in the case of the hamsters.

English, Schlack and Ellinges ('54) again used the rat as an experimental animal with an x-ray dose of 1500 r. delivered in a single dose, at 200 k.v. They reported a complete break in the incisor tooth formation, with however an apparent recovery, which sometimes resulted in the formation of an "extra" incisor tooth lateral to the first. This resulted in tooth formation in two sections; a tooth formed during the pre-exposure period, and a second incisor formed in the post-exposure period from cells which either recovered from the irradiation damage, or were so resistant to radiation damage that they escaped serious injury.

Murai, Kikuchi and Nakamura ('58) instead of using rodents as the experimental animals, based their observations on the young domestic cat. They irradiated the animals in several sessions until a total dose of 1800 r. to 2000 r. had been given. The physical factors were 160 k.v., 3 m.a., S.T.D. of 23cm, with filters of 0.5 mm. Cu and 1.0 mm. Al.

A total of 13 animals were irradiated, and sacrificed at intervals, with the longest survival period being 375 days in one case.

They reported a wavy distortion of the dental tissues in the basal portion of the tooth with cessation of histodifferentiation. The roots of the teeth appeared slender and in some cases resorption of the basal dentine occurred, along with the formation of osteodentin.

Other observations were, a lack of root formation in the molar and premolar teeth that were irradiated during their crown forming stage, occasional ankylosis and abscess formation, and the delayed eruption of the permanent dentition.

Using lead acetate as an indicator of dentin apposition in two animals, they observed a decrease in the rate of dentin apposition following irradiation. It should be noted however that no control observations were made on the rate of dentin apposition, other than the rate of dentin growth during the pre-radiation period. Moreover these observations are in contradistinction to those noted by Weinreb ('49) in the rat incisor. Using Alizarine Red S as an indicator, Weinreb was unable to distinguish any significant

difference in the rate of dentin apposition between his experimental and control group, following x-ray irradiation in the 4000 r range.

B) Effect of X-Ray Irradiation on the Temporomandibular Joint.

It is well known that x-ray irradiation of the long bones can result either in a temporary cessation of growth (Brooks and Hillsdrom '33; Heller '48) or in a complete and permanent cessation of growth both in length and in weight (Regen and Wilkin '36 and '37).

The severity of growth inhibition varies directly with the dose of x-ray radiation administered (Gates '43). It has been shown that this inhibition in growth of the long bones is due specifically to the effects of radiation on the centers of endochondral bone formation (Bisgard and Hunt '36).

Microscopic examination of the proliferating cartilage reveals alterations in the morphology of the cartilage cells, and a disorientation of endochondral ossification (Call, Lingley and Hilchen '40; Gates '43) following x-ray irradiation in the 1000-3000 r. range.

While the effects of x-ray irradiation on the growing long bones has received attention its effects on the temporomandibular joint and growth centers of the skull has been neglected to a great extent.

Burstone ('50) studied the effects of doses of 1500 r., 3000 r., and 5000 r. delivered at 50 k.v., 2 m.a., S.T.D. of 20 m.m. with no filters, on the temporomandibular

joints of mice under four days of age. He noted a hemiatrophy of the mandible which became prominent after several weeks. Mice which had received 5000 r. exhibited aplasia and fibrosis of the marrow, as well as a reduction in the number of cells in the intermediate and hypertrophic zones of the condylar cartilage. The cartilage cells showed evidence of degeneration. He also reported on the effects of P32 on the temporomandibular joint of mice, which were essentially the same as those due to x-ray irradiation, when administered in doses of from 5-60 microcuries/gm. animal weight.

Several other workers (Bruce '50; Murai, Kikuchi and Nakamura '58) have noted an apparent decrease in the growth of the jaws following irradiation, but these studies were primarily concerned with the effect of x-ray irradiation on the dental tissues, and the observations on the growth of the jaws were not substantiated either by microscopic examination of the growth centers other than the teeth, or by measurements of the skulls.

III. MATERIALS.

This study is based on the histological and roentenographic analysis of 51 kittens, of which 25 were subjected to a localized dose of 2000r. of x-ray irradiation. This was given in two doses of 1000 r. each, 7 days apart. Both sides of the skull were irradiated in an alternating fashion during each radiation session.

At the beginning of the experiment the animals were examined roentenographically in order to ascertain their stage of dental development. Only those animals in an active stage of growth were used.

Throughout the experiment they were fed ad libitum on horse meat and condensed milk.

The animals were divided into three groups (Table 1):

Group A. This group consisted of 21 experimental animals and 6 controls. The experimental animals were sacrificed at intervals of 2 months, 4 months, 8 months, 10 months and 12 months following irradiation. Demineralized sections were prepared for histologic analysis.

Group B. This group consisted of 4 experimental cats and 2 controls. The experimental animals were irradiated, and all the animals, experimental and controls, received injections of alizarine Red S at intervals. The animals were sacrificed at the end of 4 months, and the rate of dentin growth measured in ground sections of the jaws.

Group C. This group consisted of 18 adult control animals.

The skulls were sectioned in the frontal and sagittal planes, and lateral skull x-rays taken.

This control group was used as a reference, to measure the degree of growth retardation in the animals of Group A who survived to maturity.

IV. METHODS.

A. Irradiation.

1. Technique.

A Westinghouse machine was used. The machine was standardized at 110 k.v., 10 m.a. S.T.D. 20 cm., and a filter of 3 m.m. Al. The output was measured by a Victoreen x-ray dosimeter (Victoreen Instrument Co., Cleveland, Ohio).

The body of the animal was protected by a box constructed of 4 m.m. of lead, 2 m.m. of aluminum and lined with plywood. The head protruded through the box.

2. Sedation.

The animals were sedated with acepromazine. This was administered in a tablet form per os. The required dosage was about 10 mg. per 450 gms of body weight.

3. Weight.

The animals were weighed weekly during the course of the experiment.

B. Injections of Alizarine Red S.

The method described by Schour, et al ('41) was used.

A one per cent solution of alizarine Red S in 0.45 per cent sodium chloride was prepared. Alizarine Red S of color index 1034, obtained from the Hartman-Leddon Co., was used.

2.5 c.c. of this solution for each 450 grams of body weight was injected intraperitoneally 7 days prior to the date set for irradiation. Injections followed at intervals

thereafter, as follows;

Pre-irradiation period. An interval of 7 days occurred between the first and second injections. The animals were irradiated at the end of this period.

Period 1. An interval of 21 days occurred between the second and third injections.

Period 2. There were 23 days between the third and fourth injections.

Period 3. 42 days passed between the fourth and fifth injections.

Period 4. 14 days following the last injection the animals were sacrificed.

C. 1. Preparation of Ground Sections.

The animals were sacrificed by an overdose of nembutal, injected intraperitoneally. After decapitation the heads were immediately fixed in 10 per cent neutral formalin for 72 hours or more. The heads were then split with a rapidly rotating diamond wheel under a water spray, in the frontal plane at the level of the external auditory meatus. A block of bone was removed anterior to the frontal cut, so as to include the sagittal suture. Finally the anterior half of the skull was cut in the mid sagittal plane, and both halves were x-rayed on Kodak Blue Brand film. The sectioned skulls were then fixed in neutral formalin for a further period of two weeks or more.

The usual method of preparing ground sections by fixing the specimen with household cement to a cork and

then grinding it to the desired thickness was found to be inadequate for our purpose. With this method, most, if not all, of the soft tissues are lost and it is exceedingly difficult, if not impossible, to keep the normal tooth-bone relationship intact.

Since for this study it was important to retain the soft tissues and the tooth-bone relationship intact, a method of embedding in plastic was employed. The advantage of such a method lies in its simultaneous preservation of soft and highly calcified structures in their normal relationship.

Furthermore, the protection of the specimen by a rigid block of high tensile strength is a most advantageous factor in preventing fractures of the sections.

The material for embedding was prepared as recommended by the manufacturer with the modifications suggested by Bohatirchuk ('57). The resin manufactured under the trade name of Bioplastic by Ward's Natural Science Establishment Inc. was used. This has the advantage of being easy to handle, requires no complicated equipment and gives clear transparent sections.

The resin blocks containing pieces of the jaws, temporomandibular joint, or sagittal suture were mounted on the tooth sectioning machine manufactured by William E. Niclas Co., and sectioned at about 50 microns under a constant stream of water.

The sections were transferred to a standard Buehler metallurgical polisher and ground to about 30-40 microns with pumice, and polished with a fine abrasive supplied by Ward's for this purpose.

Finally, the ground sections were mounted in the usual manner under gum damar.

2. Measurement of the Rate of Growth of Dentin in Width.

The distance between the successive alizarine lines and between the last alizarine line and the pulpal contour was measured by means of a filar micrometer.

The distance between the alizarine lines produced at regular intervals represented the growth of dentin in width. The width was measured between two alizarine lines wherever the lines appeared sharp and distinct, so as to avoid measuring tangential sections. At least two series of measurements were taken for each section. All measurements were made along the dentinal tubules. A simple arithmetical average of the figures obtained for the two series, in each animal, was computed for each time interval, and expressed in microns per day.

3. Measurement of Rate of Growth of Dentin in Length.

The distance between the starting points of the different alizarine lines along the dentino-enamel junction, and root surface was measured. Owing to the slight curvature of the teeth, these linear measurements are only approximate determinations.

D. Decalcification, Sectioning and Staining.

The specimens of Group A were prepared for

histological analysis. They were fixed immediately following the sacrifice of the animal.

The skulls were then sectioned in both a frontal and sagittal plane, and lateral x-rays taken. The temporomandibular joints, the jaws, and a block of bone surrounding the sagittal suture were decalcified in 5 per cent nitric acid, until such time as x-rays no longer showed calcified structures. They were then embedded in celloidin and cut at a thickness of about 15 microns. The sections were stained with 1) Weigert's hematoxylin eosin 2) Masson Trichrome and 3) Gomori's silver impregnation.

Feline Distemper and Feline Pneumonitis Vaccine.

During the early stages of the experiment a large proportion of the animals died with a respiratory condition manifest by sneezing and coughing, and often accompanied by a serous or mucopurulent discharge of the eyes and nose. This was eventually controlled by isolation, and injections of feline distemper and feline pneumonitis vaccine. None of the animals that died of these contagious diseases was included in the final experimental groups.

E. Post Radiation Skull X-Rays.

After the initial fixation, the skulls were sectioned in the frontal and sagittal planes and lateral skull x-rays taken with a General Electric dental machine calibrated at 75 k.v. and 10 m.a., with an exposure time of 2/10 of a second. The distance between the x-ray tube and the film was 5.5 feet.

1. Anatomic Points of Reference used in Measurements of Lateral Skull X-Rays (Fig. 1).

Measurements were taken of the lateral skull x-rays of the animals in Group C, and those animals in Group A with a survival period of 6 months or more. The following reference points were used.

Point T. The most rostral point on the surface of the mandibular condyle.

Point M. The most anterior point on the bony surface of the mandible.

Point G. A line was drawn along the lower border of the mandible and extended to meet at right angles, a line drawn from the most rostral point on the surface of the mandibular condyle (point T). The intersection of these two lines was the point of reference.

Point N. The most anterior point of the nasal bone.

Point A. The junction between the mesial surface of the first maxillary molar tooth, and the alveolar process of the maxilla.

The following measurements were taken using a needle point compass and a Boley gauge.

1. Between points T and M.
2. Between points N and A.
3. Between points T and G.

Measurements were taken of the films of both sides of the skull, and a simple arithmetical mean determined.

V. FINDINGS.

A. General Effects.

1. Weight.

The weight of the animals increased during the first two weeks of the experiment. During the third or fourth week, most of the animals showed a levelling off, or a slight decrease in weight. By the sixth week they had resumed their increase in weight.

B. Local Effects.

1. Depilation.

By the third or fourth week all of the irradiated animals showed some loss of hair around the head. In some instances this was patchy, in others it resulted in a nearly complete loss of hair. By the tenth week there was a secondary growth of white hair in the denuded areas (Fig. 2).

2. Ophthalmologic Signs.

Clouding of the cornea developed in one or both eyes, in about 25% of the animals.

C. Microscopic Findings: Teeth.

I. - Rate of Growth of Dentin, as Measured by Alizarine Red S.

The rate of growth of dentin was measured in ground sections of jaws of animals which had been given injections of alizarine Red S at intervals.

1. Pattern of Dentin Growth.

In the control animals, the basal end of the dentin diminished gradually and uniformly in thickness until it

tapered to a very thin edge. In longitudinal sections through the center of the teeth, the alizarine lines started at intervals in the dentin of the crown, and ran in parallel lines toward the apical end (Fig. 3). The first line, and in some instances the second line ended at the dentinoenamel junction. The third, fourth, and fifth lines ended on the root surfaces of the teeth.

Whereas, in the normal animal the basal end of the dentin wall terminated in an edge, in the irradiated animal the dentin showed a bluntness at the basal end, which increased in width with the increase of the post irradiation period. Moreover the third, fourth, and fifth alizarine lines rather than ending at intervals on the root surface as in the controls, ended adjacent to one another, at the apical end of the tooth (Figs. 4 and 5). The alizarine lines remained parallel, as in the controls, except at the apex where they converged.

2. Rate of Growth of Dentin in Width.

Measurements of the distances between the successive alizarine lines showed that the average growth of dentin in width in the controls and the experimental animals before irradiation was 6.6 microns per day. After irradiation, the daily rate of growth of dentin in width averaged 5.7 microns for the first period, 6.7 microns for the second period, and 5.7 microns and 4.3 microns for the third and fourth periods respectively.

The comparable rates of growth for the control

animals were 6.0 microns for the first period, and 6.9, 6.8 and 5.7 microns for the subsequent periods. A comparison of the control and irradiated animals shows that the rate of growth in width of the dentin was minimally affected by irradiation (Tables 2 and 4).

3. Rate of Growth of Dentin in Length.

The measurements between the terminal points of the successive alizarine lines showed the most marked changes. The control animals showed an average growth in length of 21 microns per day for the first period, 28 microns in the second, and 26 and 16 microns in the third and fourth periods.

The irradiated animals showed an average growth of 13 microns per day for the first period, 12 microns for the second, and third periods, and 3 microns for the final period. The growth in length during the pre-irradiation period was not measured because the terminal points of the first alizarine line were not visible.

A comparison of the irradiated animals with the controls indicates a reduction to 61 per cent of the growth of the control animals for the first period. In the second and third periods, there was a reduction to 42 per cent of the rate of growth of the control animals for the same period and to 18 per cent for the final period (Tables 3 and 4).

II. Morphological Findings

1. Enamel.

No changes were noted in the enamel formed either

before or after the experimental procedure, when examined in ground sections and routine celloidin sections. The enamel matrix was of normal shape and form.

2. Dentin.

The dentin of the crown formed either before or after the experimental procedure showed no abnormal changes, calcification was normal, and the predentin was of normal width. Abnormalities of dentin growth were only seen in the root portion of the teeth.

Two months following irradiation the dentin toward the apex showed a blunted end. A primitive type of calcified tissue was in some instances associated with the tip of the dentin. This tissue, osteodentin, showed cell inclusions, but it was not characterized by the normal, lamellar pattern of adult bone, or the tubular pattern of dentin (Fig. 6).

Four months following irradiation there were increased amounts of osteodentin in the apical areas. This was frequently associated with centers of basophilic dystrophic calcification (Figs. 7, 8 and 9). The argyrophilic fibers in the osteodentin were arranged in an irregular fashion, as compared to that seen in bone or dentin (Fig. 10).

Eight, ten and twelve months. During this period the pulpal floor, or the apical ends of the teeth depending on their stage of development were completely sealed by osteodentin and regions of dystrophic

calcification. This phenomenon was noted in all of the animals surviving eight months or more. Here and there a vascular channel could be seen traversing the sealing bridge of osteodentin.

3. Pulp.

Two months following irradiation the pulp showed signs of hyperemia and oedema. There was a lack of differentiation of the odontoblasts at the basal end of the tooth, and a great reduction in the number of cells of the proliferating zone of the pulp. The epithelial diaphragm and epithelial root sheath in the developing teeth were no longer evident. (Fig. 11)

Four months following irradiation no dramatic changes were noted in the pulp, except for areas of haemorrhage which appeared to be fresh. Some cases showed areas of lightly staining, granular, eosinophilic material, in the center of the pulp, however this finding was variable and was noticed in a few control animals.

Eight, ten and twelve months. The pulp showed extensive areas of dystrophic calcification frequently associated with haemorrhage. These areas of calcification had an oblong or round shape and were arranged parallel to the dentinal wall (Figs. 8 and 9). They stained deeply with hemotoxylin, and no organized structure could be seen. As a rule the periphery of these islands of calcified tissue were of a fibrillar nature, and stained lightly with eosin. In some instances the areas

of calcification were incorporated into the dentinal wall by the growing dentin.

This phenomenon was seen in 78% of the irradiated animals that survived eight months or more, and was observed in one of the control animals.

4. Periodontal Membrane.

There was a decrease in the width of the periodontal ligament in some areas, with the fibers running parallel to the root surface, rather than at an acute angle as in the controls. Frequently, the periodontal membrane was completely obliterated with a resultant ankylosis.

(Fig. 12). Ankylosis of some teeth was found in 42% of the irradiated animals. Both the permanent and retained deciduous teeth showed varying degrees of ankylosis. All of the animals that showed ankylosis of some teeth had survival periods of eight months or more. No evidence of ankylosis was found in any of the control animals.

Resorption of the cementum and dentin was a frequent phenomenon in teeth showing ankylosis. It varied in degree from a slight resorption of the root surface in one area, to an extensive resorption and tunnelling of the whole root (Fig. 13).

All of the animals, both the controls and the experimental groups, showed an inflammatory reaction in the region of the attached gingiva. This seemed to be more intense in the experimental groups, particularly in animals that survived eight months or more.

The inflammatory reaction of the attached gingiva was

frequently associated with a resorption of the root in the region of the epithelial attachment (Fig. 14). These areas showed dense collections of argyrophilic fibers. Some of the fibers could be seen running from the dentin into the resorption lacuna (Figs. 15 and 16). The degree of resorption was variable. At its extreme the inflammatory resorption extended from the region of the attached gingiva into the pulp of the tooth. In some cases this was associated with the deposition of bone along the pulpal wall. This bone was either deposited on dentin, or it replaced dentin previously resorbed.

This association of inflammation and lateral root resorption was observed in 75% of the irradiated animals that survived four months or more. It was not seen in any of the control animals.

Most of the animals that showed lateral root resorption and inflammation, showed some degree of inflammation in the transseptal and alveolar crest fibers of the periodontal ligament.

The formation of shallow pockets, associated with debris in the gingival crevice was a frequent finding in the irradiated animals with a survival period of 8 months or more.

D. Roentgenographic Findings: Teeth.

The roentgenograms of the irradiated animals, showed the following changes. The basal end of the teeth were

blunted, which resulted in teeth without roots, or in teeth with short stubby roots. The roots of the canines were decreased both in length and in width. This resulted in canines which were slender in appearance as compared to the prominent canines of the controls (Figs. 17 and 18).

The apical regions of the incisor teeth of irradiated animals surviving eight months or more showed a circumscribed, radiolucent area, when the lateral skull x-rays were viewed (Fig. 18). This was seen in 88% of the experimental animals surviving eight months or more, but was not observed in the controls. These areas were associated with a resorption of the incisor teeth in some cases.

In 33% of the experimental animals with a survival period of four months or more some of the permanent molar teeth remained embedded in the jaw (Fig. 19). In some instances the teeth on one side of the jaw were embedded, while on the opposite side they had erupted. The deciduous teeth in the cases of embedded permanent teeth were retained.

E. Rate of Eruption.

Irradiation resulted in a decreased rate of eruption of the molar teeth. They tended to remain embedded in the jaws for a longer time than usual (Fig. 19). However in the majority of cases the molar teeth erupted into the mouth, despite the lack of root formation.

F. Microscopic Findings: Temporomandibular Joint.

No morphological differences were noted microscopically between the joints of the control and experimental animals.

The width of the cartilage plate in both the control and the experimental animals was measured. There was no statistical difference in the width of the cartilage plate in the two groups.

G. Microscopic Findings: Sagittal Suture.

No morphological difference was noted either in the bony tissue, or the connective tissue in the suture.

H. Appositional Growth of Bone Tissue of the Jaws.

No changes could be seen in the bony tissue of the jaws. Bone growth, as evidenced by osteoid formation was frequently seen. Successive alizarine lines both in the alveolar bone and the body of the mandible indicated continued bone apposition (Fig. 20).

1. Effect on the Growth of the Facial Skeleton

Measurements of the distances between specific anatomic points on the lateral skull x-rays of the control animals (Fig. 1) showed that the average distance between the points T and G was 1.13 m.m., between the points T and M 5.37 m.m., and between the points N and A 2.05 m.m. Similar measurements in the irradiated animals showed distances of .94 m.m. between the points T and G, 4.63 m.m. between T and M, and 1.73 m.m. between N and A. This represents a reduction to 83% of the control animals in the first instance, and to 86% and 84% in the latter (Table 5).

VI. DISCUSSION.

A. Growth of Dentin in Length.

The earliest signs observed following the irradiation of the animals were the degeneration of the connective tissue cells and the appearance of oedema in the proliferative zones adjacent to the odontogenic epithelium.

The fact that the first injury following irradiation appeared in the proliferative zone of the teeth can be explained in the following manner: It has been frequently observed that the least differentiated cells in, or immediately preceeding mitosis, are specially radiosensitive (Bloom '48). The proliferative zone of the pulpal connective tissue in the growing tooth consists of young and undifferentiated connective tissue cells, which are adjacent to the epithelial cells of Hertwig's root sheath. Both these tissues undergo mitotic division frequently.

In the normal development of the growing tooth, the inner enamel epithelium exerts an organizing influence on the adjacent connective tissue cells of the dental pulp, which differentiate into odontoblasts. The odontoblasts then start to form dentin, and the ameloblasts the enamel matrix:

Following the experimental procedure, the odontoblasts in the proliferative zone of the pulp continued to differentiate and to form dentin for a period of time, after which dentin formation and the complete differentiation of the odontoblasts ceased. The odontoblasts which had differentiated before the irradiation, continued to form dentin at a nearly normal rate.

What little further growth in length occurred was due to the formation of a less specialized tissue, osteodentin. The reaction of the growing teeth to irradiation varied with the stage of development of the tooth. Teeth that were irradiated during the crown forming stage, continued their longitudinal growth, until the completion of the crown before growth ceased. Whereas teeth that were irradiated during the root forming stage, showed a cessation of growth in length shortly after the experimental procedure.

The continued growth of teeth irradiated during the crown forming stage is probably explained by the influence of the enamel epithelium on the pulpal tissues, and is in accord with the findings of the effects of irradiation on the labial surface of the rat incisor (Weinreb ('49)). When the level of x-ray irradiation is increased to sufficiently high doses, growth of the teeth can be stopped during the crown forming stage (Gowgiel '61).

B. Growth of Dentin in Width.

It is a well known fact that the more differentiated, or specialized cells tend to be more radioresistant than less differentiated cells. This probably accounts for the fact that no statistical difference between the control and experimental animals was noted during the first, second and third periods, while during the fourth period a significant difference in the rate of dentin growth in width was observed. This difference during the latter period may be due to a cumulative effect, which was not evident during the earlier part of the experiment. These findings differ with Murai, Kikuchi and Nakamura ('58)

observations that growth in width is temporarily reduced following irradiation at the 2000 r. level, and then gradually returns to a nearly normal rate of growth.

The difference in our observations and those of the Japanese workers may be explained by the controls used in the two studies. Murai and his co-workers used the pre-irradiation period of their experimental animals as the control for their measurements of the growth of dentin in width following irradiation. However the rate of growth of dentin in width varies from time to time in the normal animal (Table 2). This fact may explain the temporary decrease of growth in width, which they reported following irradiation.

C. Eruption of Teeth.

Several "forces" have been suggested to account for the eruption of teeth. Among those that have been considered are: the growth of dentin, the proliferation of pulpal connective tissues which generate pressure against the cushioned hammock ligament (Sicher '42), the degree of vascularity of the periapical tissues (Massler and Schour '41), the pressure from muscular action, the apposition and resorption of bone, the readjustment of the periodontal ligament (Taylor '50), and the growth of the tooth follicle (O'Brien, Bhaskar and Brodie '58).

In the experimental animals, most of the partly formed teeth whose growth had been interrupted by irradiation erupted into the mouth. This indicates that while the growth of the root, and proliferation of pulpal tissues may be a factor in the eruption of teeth, they are not essential to it.

Furthermore it would seem that these factors may not be the principal "forces" responsible for tooth eruption. Osteoid formation was observed adjacent to partly erupted, rootless teeth. This may account in part for their eruption.

D. Growth of Bone Tissue.

Both endochondral type bone growth at the temporo-mandibular joint, and bone apposition along the alveolar crest and surfaces of the jaws continued following irradiation.

The dimensions of the jaws of the irradiated animals were less than that of the control animals. This may be due either to a decrease in the rate of bone formation, or a premature cessation of the growth of the jaws, or to a temporary interruption in the growth of the jaws. However bone apposition can be shown to have occurred following irradiation, as indicated by the alizarine lines in the jaws, and the deposition of osteoid adjacent to erupting teeth. It is therefore likely that the rate of bone deposition was decreased, rather than the time. This might be explained by a damping of the enzyme systems of the osteoblasts.

It is well known that x-ray irradiation can inactivate enzyme systems in vitro (Hevesy '50). That the enzymes of bone cells in vivo can be affected by irradiation, has been shown by the work of Rogen and Wilkins ('36), who observed a decrease in alkaline phosphatase activity in previously irradiated bone. This was substantiated by Cohn and Gong ('53) who reported a decrease in the serum alkaline phosphatase, following localized irradiation to the leg of rats.

E. Growth of the Jaws.

Growth of the face in the anterior posterior direction depends on a coordinated growth of the maxilla and mandible. This is achieved in the maxilla by the interstitial growth of the connective tissue at the sutures, more particularly at;

1. the frontomaxillary suture
2. the zygomaticomaxillary suture
3. the zygomaticotemporal suture, and
4. the pterygopalatine suture.

Growth in these sutures results in a downward and forward growth of the maxilla.

The downward growth of the maxilla is also dependent on the apposition of bone on the inferior surface of the alveolar process and on the eruption of the teeth.

Proliferation of the condylar cartilage results in the growth in height of the mandibular ramus, and in the overall length of the mandible. A preponderance of bone apposition along the posterior border of the ramus, and resorption along the anterior border, are responsible for the growth of the body of the mandible in humans.

The apposition of bone along the free border of the alveolar process, and the eruption of the teeth are factors responsible for the height of the body of the lower jaw, that is the distance between the lower border of the mandible and the superior surface of the alveolar process.

The experimental animals showed a definite decrease in the dimensions of their jaws as compared to the controls.

Murai, Kikuchi and Nakamura ('58) have suggested that any decrease in the growth of the jaws, may be due to the reduced dimensions, and decreased rate of growth of the irradiated permanent teeth. However, a comparison of the measurements, shows that the dimensions of the jaws were reduced not only in the tooth bearing areas, but also in areas of the jaws that do not support teeth (TG). It would seem then, that the decreased size of the jaws is due to an actual decrease in bone growth both appositional and endochondral, rather than a decrease in the growth or size of the teeth. Furthermore in clinical cases of complete congenital anodontia, the dimensions of the jaws, with the exceptions of the alveolar processes, are within normal limits (Sarnat, Brodie and Kubacki '53), which suggests that the development of the teeth is not essential to the growth of the jaws.

F. Lateral resorption of the Incisor Teeth.

Inflammation of the gingiva was noted in all of the experimental animals, and in most of the controls. The inflammation was more intense in the experimental group. A majority of the irradiated animals showed a lateral resorption of some incisor teeth, in the region of the gingival attachment, associated with an intense inflammatory reaction.

This phenomenon has not been reported to date in any other experimental studies listed in the literature. However a form of cervical caries, has frequently been reported as occurring in clinical cases following radiation therapy in adults.

None of the cases observed in this series showed the

bacterial invasion seen in clinical caries. The resorption was associated with osteoclastic activity, and extended both above and below the gingival attachment, by means of resorption lacuna in the dentin. The inflammatory reaction seen in conjunction with this resorption, was more intense than that seen in the gingiva attached to teeth which did not show resorption. It may be, that the intense inflammatory reaction of the gingiva stimulates osteoclastic resorption of the teeth. This relationship between resorption of teeth and an inflammatory reaction is occasionally seen in clinical cases, and is a well known phenomenon in bone.

Traumatic occlusion can be rejected as a probable cause, because of the incidence of resorption in the experimental group.

The occurrence of an intense inflammatory reaction in the gingiva adjacent to the incisors is due in part to local factors, which caused a mild inflammatory reaction even in the control animals. Aptyalism due to the irradiation of the salivary glands, and the direct action of the x-rays on the mucosa accounts for the more intense inflammatory reaction in the experimental animals.

VII. SUMMARY AND CONCLUSIONS

The effects of a localized x-ray exposure of 2000 r. on the jaws of kittens was studied in 25 domestic cats of about 3 months of age.

The histologic changes in the jaws, temporomandibular joint and sagittal suture were examined in 21 experimental animals, which were sacrificed at intervals of 2 months, 4 months, 8 months, 10 months, and 12 months, following irradiation; 6 non-irradiated animals served as controls.

The changes in the rate of growth of the teeth in width and length was studied in 4 irradiated animals and compared to the results of 2 non-irradiated animals by means of vital staining with alizarine Red S.

A comparison of the dimensions of the jaws, as seen in lateral skull x-rays of 10 irradiated animals surviving 8 months or more, and 18 adult control animals was made.

The findings were as follows:

1. Differentiation of young odontoblasts from the pulpal connective tissue stopped following irradiation. This resulted in the eventual cessation of the growth in length of dentin, and the deposition of a less specialized tissue, osteodentin.
2. The odontoblasts which had differentiated prior to irradiation showed a gradual decrease in the rate of dentin formation following irradiation.
3. Ankylosis and resorption of some of the permanent and deciduous teeth was observed.
4. Despite the lack of growth in length of the

teeth, and the lack of mitotic activity in the proliferative zone of the pulp, the teeth nevertheless erupted into the mouth in a majority of cases.

5. An intense inflammatory reaction of the gingiva associated with a lateral resorption of dentin, was noted in the incisor teeth.

6. A statistically significant reduction in the growth of the jaws in the irradiated animals occurred. This was seen in both the odontogenic, and nonodontogenic areas of the jaws.

7. No histologic changes were noted in the temporo-mandibular joint, or sagittal suture.

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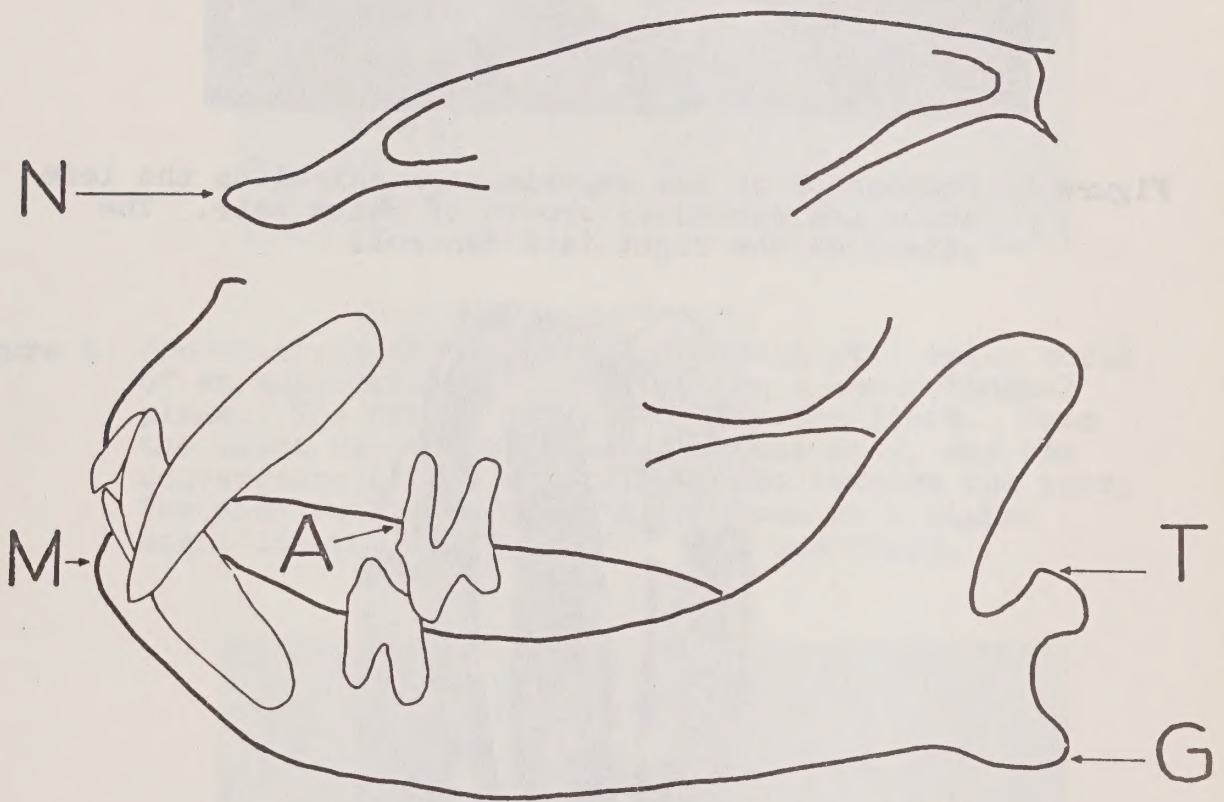


Figure 1. Tracing of a normal adult cat skull, with the landmarks used in the measurements of the jaws of the control and the experimental animals indicated. The measurements were made between the points:

- T and G, which served as an index of vertical growth,
- T and M, which was a measurement of longitudinal growth,
- N and A, which was a measurement across a tooth bearing area of the jaws.

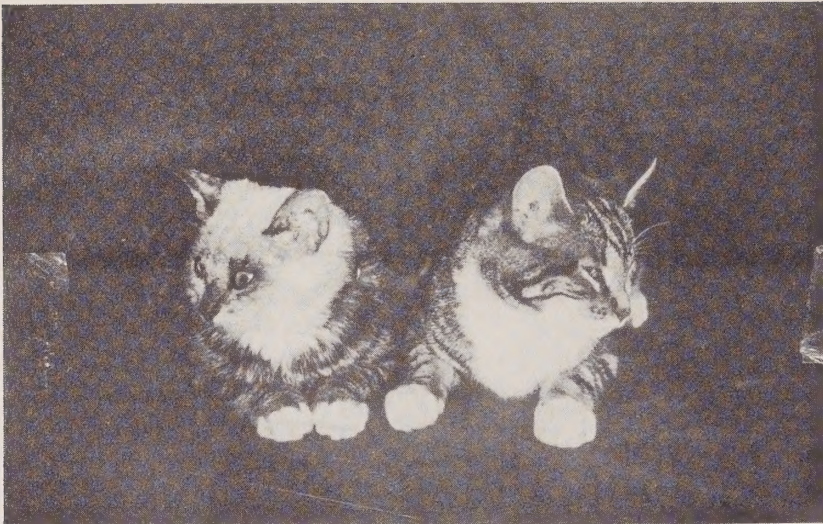


Figure 2. Photograph of the experimental animal on the left shows the secondary growth of white hair. The animal on the right is a control.

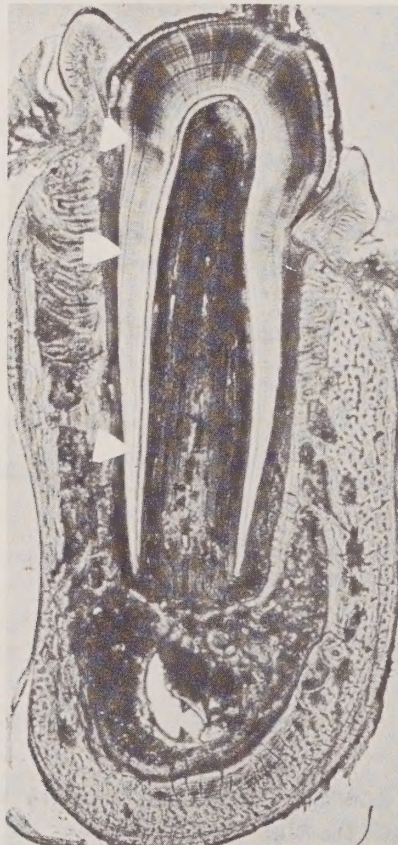


Figure 3. Photomicrograph of a ground section of a molar tooth of a control animal, cut in a bucco-lingual plane. The arrows indicate the alizarine lines in the dentin. Note the feather edge of the dentin towards the apex. X12 unstained.

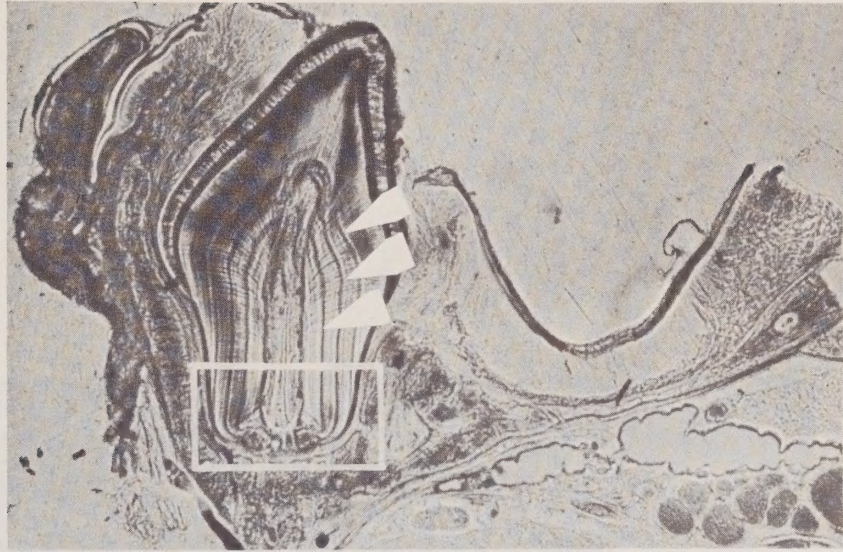


Figure 4. Photomicrograph of a ground section of a molar tooth of an experimental animal, cut in a bucco-lingual plane. The arrows indicate alizarine lines. Note the blunt edge of the dentin at the apex, and the convergence of the alizarine lines towards the apex. The area outlined in white is shown at a higher magnification in Figure 5. X12 unstained.

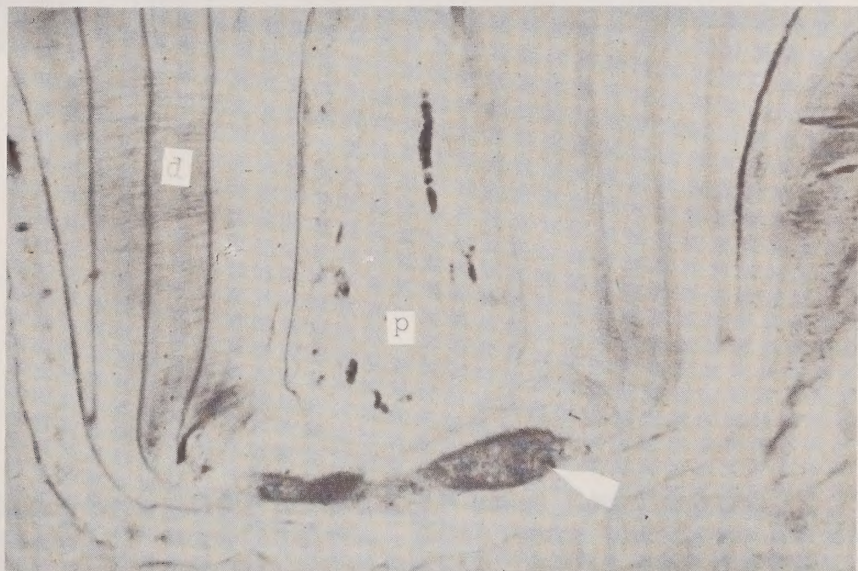


Figure 5. Photomicrograph of the area outlined in white in Figure 4. Note the convergence of the alizarine lines in the dentin (d) toward the apex. The arrow points to osteodentin which has been stained by the alizarine, p indicates the pulp. X25 unstained.

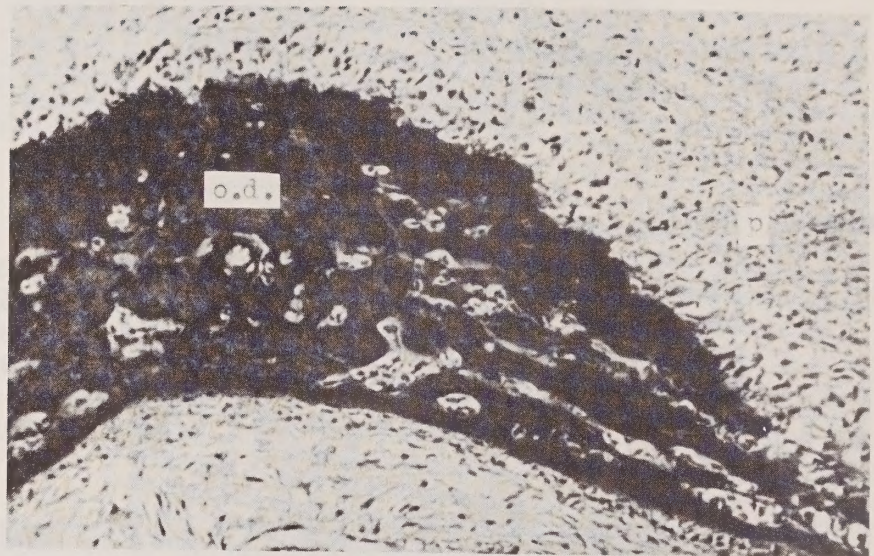


Figure 6. Photomicrograph of osteodentin (o.d.) formation at the bifurcation of a molar tooth, two months following irradiation. Note the lack of polarity of the pulp (p) cells adjacent to the osteodentin. X25 hematoxylin and eosin stain.

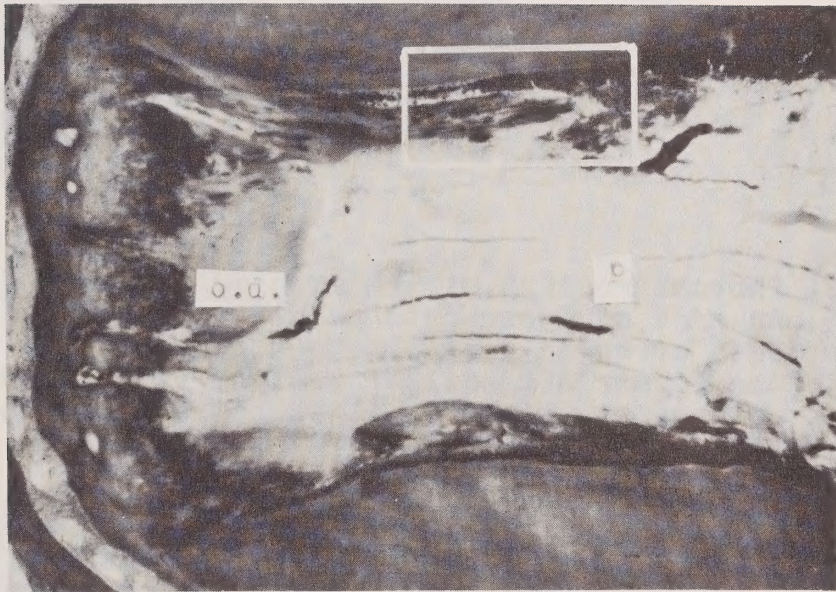


Figure 7. Photomicrograph of a molar root four months following irradiation. Note that the apex is sealed with a bridge of osteodentin (o.d.) and darker staining areas of dystrophic calcification. Tongues of calcified tissue extend along the pulpal (p) walls. The area of calcified tissue outlined in white is shown at a higher magnification in Figure 8. X25 hematoxylin and eosin stain.

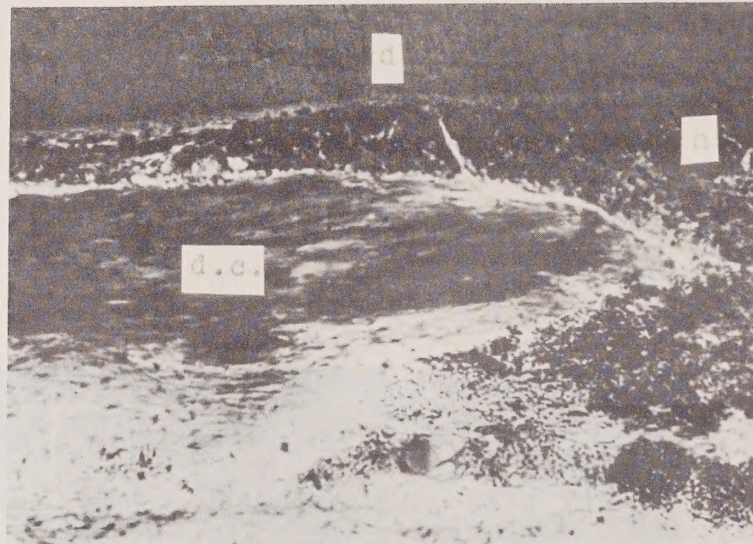


Figure 8. Photomicrograph of the area outlined in Figure 7. An area of dystrophic calcification (d.c.) can be seen arranged parallel to the dentin (d) wall, note the former's fibrillar periphery. h, indicates an area of haemorrhage. X100 hematoxylin and eosin stain.

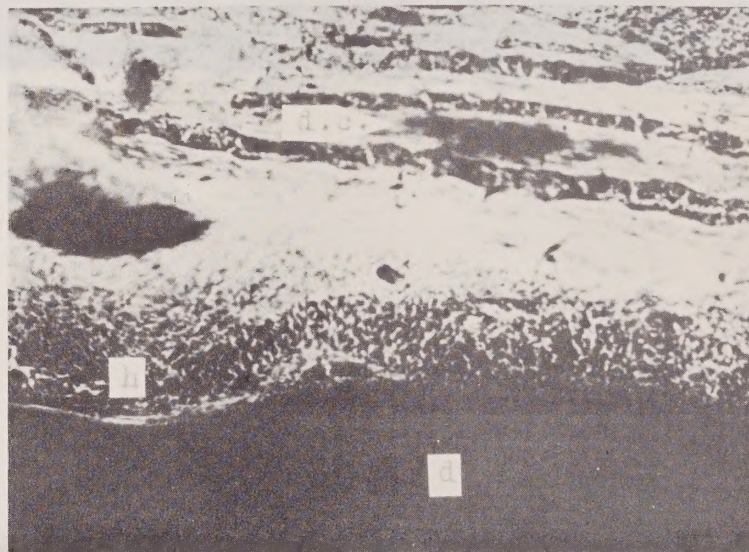


Figure 9. Photomicrograph of an area of initial dystrophic calcification, indicated by the arrow, between two blood vessels. The legends are the same as in the previous figure. X100 hematoxylin and eosin stain.

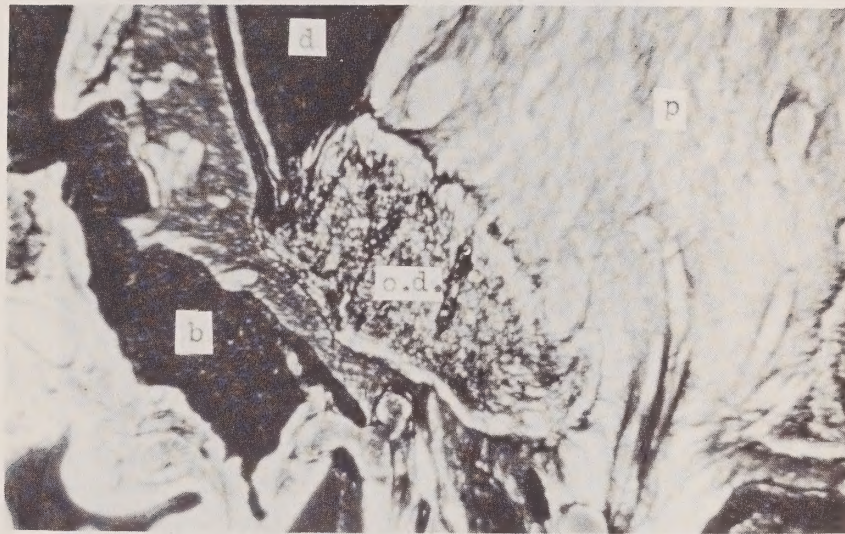


Figure 10. Photomicrograph of the apical area of a molar tooth. Note the difference in the intensity of staining and the arrangement of the argyrophilic fibers in the bone (b), dentin (d), and osteodentin (o.d.), p, indicates the pulp. X100 silver stain.

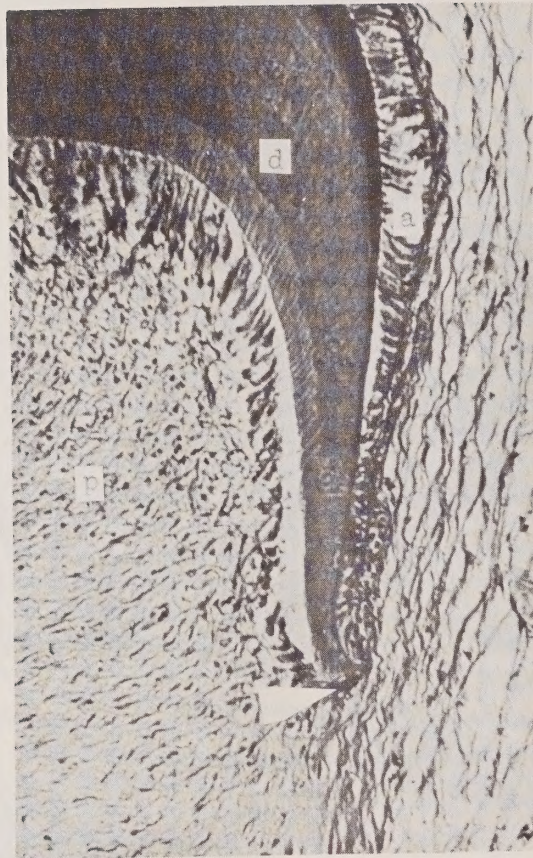


Figure 11. Photomicrograph of the crown of a molar tooth one month following irradiation. a, indicates ameloblasts; d, dentin; and p, the pulp. Note the loss of the epithelial diaphragm and the lack of histodifferentiation at the tip of the arrow. I25 hematoxylin and eosin stain.



Figure 12. Photomicrograph showing areas of ankylosis (ank.), and resorption (r.), in relation to an incisor tooth, eight months after irradiation. X25 hematoxylin and eosin stain.

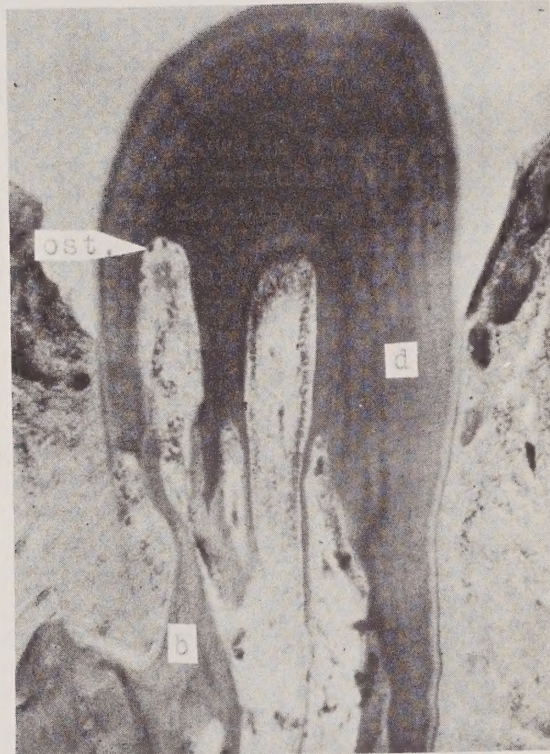


Figure 13. Photomicrograph, showing resorption of a permanent incisor tooth eight months following irradiation. The resorption is mostly in a vertical direction, and there is only a slight inflammatory reaction. There is ankylosis of bone (b) and dentin (d). Osteoclastic activity (ost.) may be seen. X25 hematoxylin and eosin stain.



Figure 14. Photomicrograph of an area of lateral resorption (r.) associated with an intense inflammatory reaction, eight months following irradiation. Note that the resorption extends directly into the pulp (p). An area of ankylosis (ank.) is also shown. X25 hematoxylin and eosin stain.



Figure 15. Photomicrograph of an area of lateral resorption (r) similar to that seen in Figure 14. Note the argyrophilic fibers running from the dentin (d) into the area of resorption. p, indicates the pulp. The area outlined in white is shown at a higher magnification in Figure 16. X25 silver stain.



Figure 16. Photomicrograph of the area outlined in white in Figure 15. Note the argyrophilic fibers streaming from the dentin (d) into the area of resorption (r.). X100 silver stain.

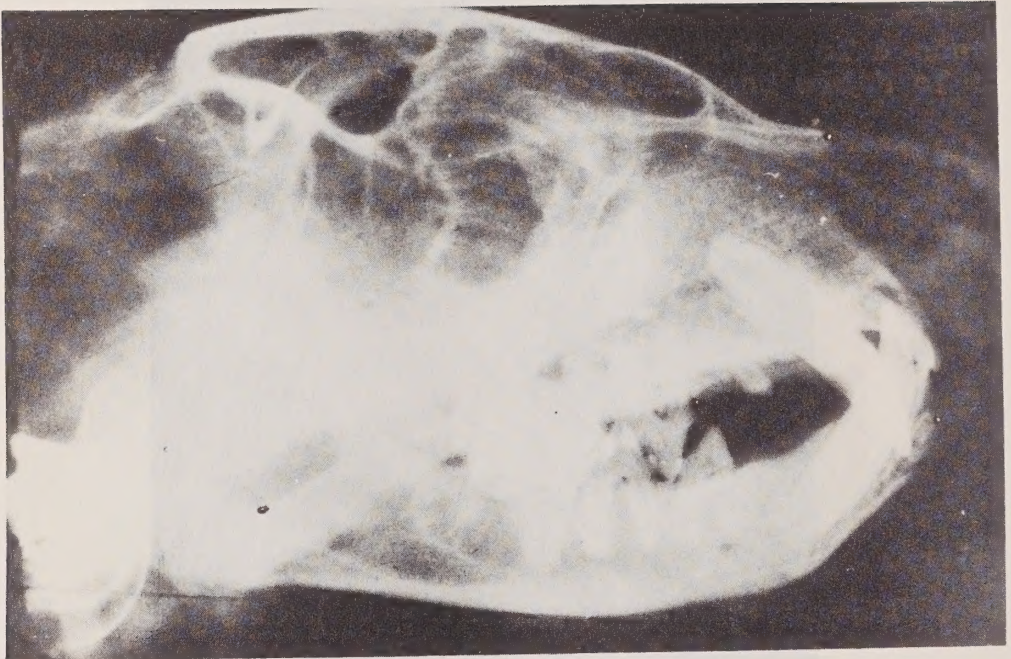


Figure 17. Reproduction of a lateral skull x-ray of a control adult animal. Notice the length and width of the roots of the teeth.

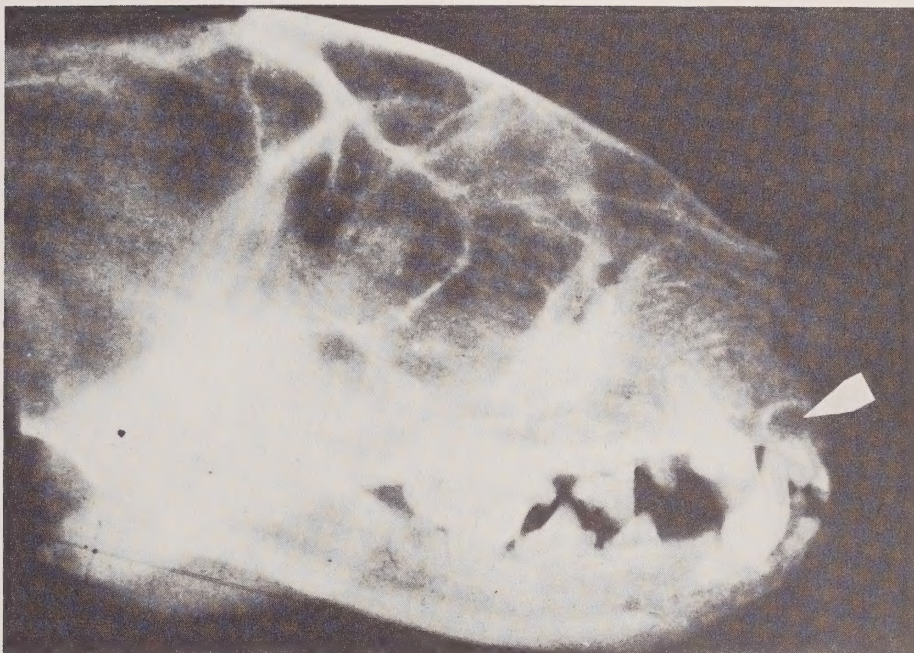


Figure 18. Reproduction of a lateral skull x-ray of an experimental animal, twelve months following irradiation. Notice that the molar teeth have erupted into the mouth despite the lack of root formation. The root of the canine is smaller in length and in width than normal. The arrow indicates a radiolucent area at the apex of the upper incisors. Lateral resorption of the incisors was observed in this animal.

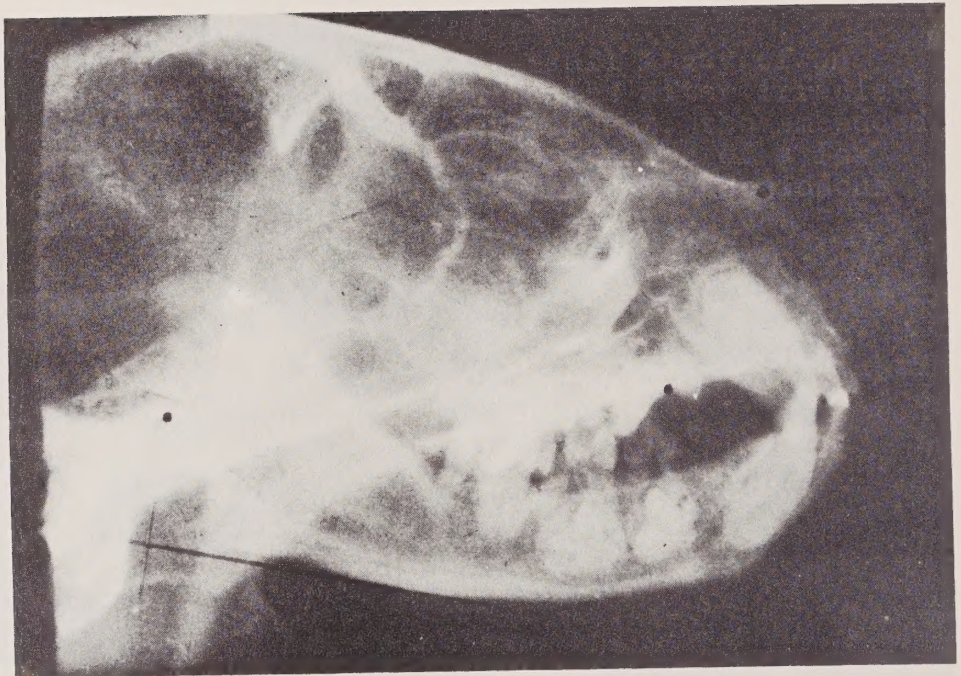


Figure 19. Reproduction of a lateral skull x-ray of an experimental animal, ten months after irradiation. Note the lack of root development, the embedded permanent molar teeth, the presence of a few deciduous molar teeth and the upper deciduous canine.



Figure 20. Photomicrograph of a ground section of alveolar bone adjacent to a molar tooth, showing the alizarine lines, indicated in red. X25 unstained.

TABLE 1EXPERIMENTAL HISTORY OF THE ANIMALS IN EACH GROUP

<u>Length of Experiment</u>	<u>Number of Animals</u>					
	<u>Group A</u>		<u>Group B</u>		<u>Group C</u>	
	<u>Exp.</u>	<u>Control</u>	<u>Exp.</u>	<u>Control</u>	<u>Exp.</u>	<u>Control</u>
12 months	3	2	-	-	-	18
10 months	3	1	-	-	-	-
8 months	3	1	-	-	-	-
4 months	4	1	4	2	-	-
2 months	8	1	-	-	-	-
	—	—	—	—	—	—
TOTAL	21	6	4	2	0	18
	==	=	=	=	=	==

TABLE 2.

RATE OF GROWTH OF DENTIN IN WIDTH, IN THE ANIMALS OF GROUP B.
(u/day)

<u>Period</u>	<u>Animals</u>							
	<u>Experimental</u>				<u>Control</u>			
				Mean				Mean
Pre-Irradiation	6.3	6.8	7.0	6.5	6.6	7.0	6.0	6.5
I	6.3	5.1	6.0	5.5	5.7	7.0	5.1	6.0
II	7.0	-	6.8	6.4	6.7	7.2	6.7	7.0
III	5.2	6.0	5.7	5.9	5.7	6.3	7.3	6.8
IV	3.9	4.0	4.2	5.1	4.3	4.6	6.8	5.7

The significance between the experimental and control groups was calculated using the t test, the result was found to be significant at the 5% level. For further accuracy the animals in period IV were compared, the difference was significant at 5% level, however there was no significant difference when comparing the animals in groups I, II and III.

(Expressed as Percentage Difference $\frac{P - C}{C} \times 100$).

The reduction in growth of dentin in width is statistically significant at the 5% level ($P < .05$) during the fourth period, but not during the first, second and third periods.

The reduction in growth of dentin in length is statistically significant at the 1% level ($P < .01$).

TABLE 3.

RATE OF GROWTH OF DENTIN IN LENGTH, IN THE ANIMALS OF GROUP B.

(u/day)

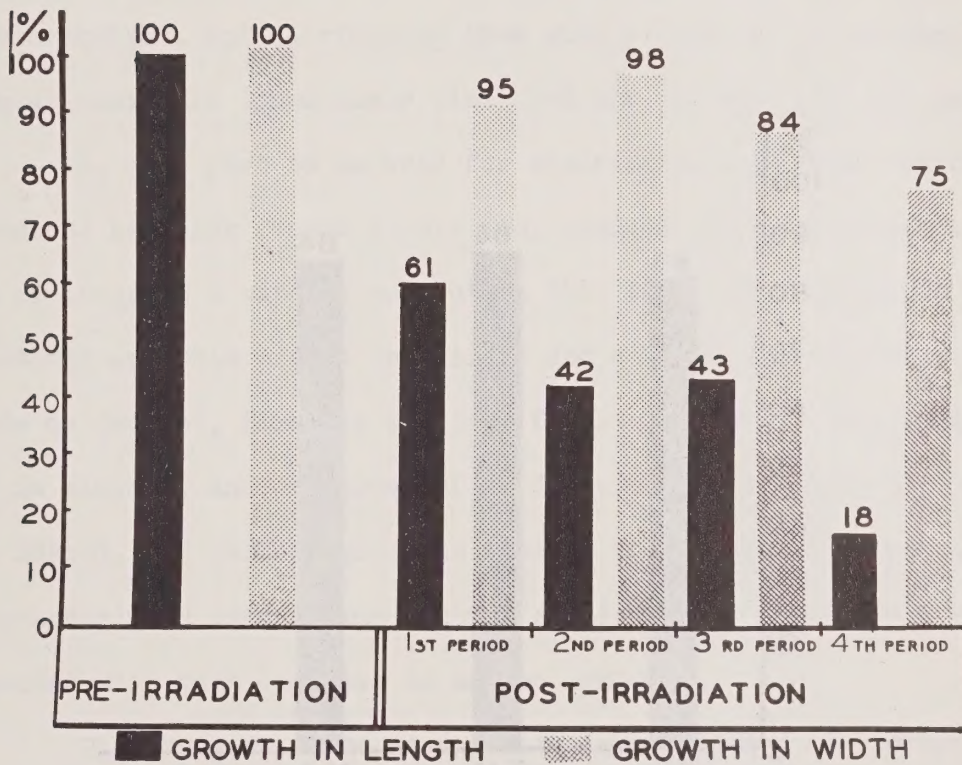
<u>Period</u>	<u>Experimental</u>					<u>Control</u>		
					Mean			Mean
Pre-Irradiation	-	b -	-	-	-	-	-	-
I	12	14	12	13	12.7	30	19	24.5
II	13	16	10	12	12.7	29	26	27.5
III	20	7	9	13	12.2	26	26	26
IV	5	4	6	0	3.7	12	19	15.5

The significance between the experimental and control groups was calculated using the t test,* the result was found to be significant at the 1% level. For further accuracy the animals in period IV were compared, the difference was again found to be significant at 1% level.

$$* t = \frac{\bar{X}_i - \bar{X}_j}{\sqrt{s^2 \left(\frac{1}{n} + \frac{1}{n} \right)}}$$

TABLE 4.

CHANGES IN THE RATE OF GROWTH OF DENTIN IN LENGHT AND WIDTH
FOLLOWING IRRADIATION WITH A DOSE OF 2000 R.



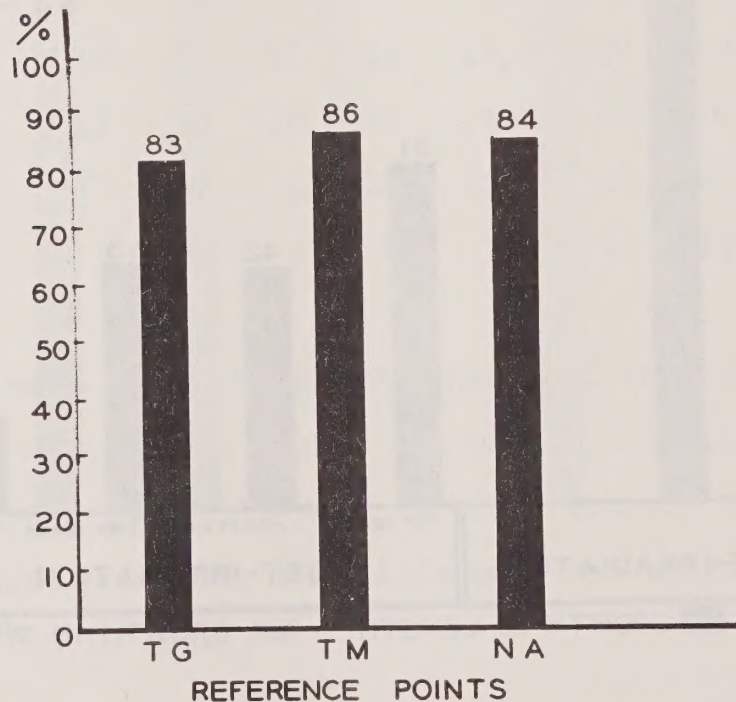
(Expressed as Percentage Differences from the Normal: normal 100%).

The reduction in growth of dentin in width is statistically significant at the 5% level ($P < .05$) during the fourth period, but not during the first, second and third periods.

The reduction in growth of dentin in length is statistically significant at the 1% level ($P < .01$).

TABLE 5.

MEASUREMENTS OF THE GROWTH OF THE SKULL OF KITTENS FOLLOWING
IRRADIATION WITH A DOSE OF 2000 R.



(Expressed as a percentage difference from the Normal: normal = 100 percent).

The result is statistically significant at the 1% level ($P < .01$).

APPENDIX - D

W. B. Donohue - DA-100

Report on Project D-100

A. METHODS

The cryostat (International-Harris cryostat) was received in June and set up in the laboratory. With the assistance of Monsieur Gérard Albert, a dental student on a summer grant from the N.R.C., the technique for handling and cutting the tissues was evolved. Briefly this consists of fasting the animals overnight, and sacrificing them with a blow to the cervical spine. The buccal mucosa is immediately dissected and divided into two parts.

1. The part to be used for histological sections (qualitative measurement) is quick frozen in dry ice, mounted in the cryostat using blocks of liver as a support and cut in this fresh state at about 10-15 μ . The sections are then placed on a slide and quickly immersed in cold (-73°C), "liquide de Gendre", fixative solution for 12-24 hours. The sections are then washed in alcohol, and one group of slides are stained using the Hotchkiss P.A.S. method, and another group is stained using the Best Carmine stain. No counterstain is used in the routine sections. In both groups some slides are treated with malt diastase to act as controls.

2. The part to be used for the quantitative measurement is scraped with a scalpel to remove muscle tissue, until a thin membrane of epithelium and connective tissue remain. It is then quick frozen in dry ice, weighed and the glycogen measurement carried out using the methods of Walaas and Walaas (J. Biol. chem. 187:769) and Nelson (J. Biol. chem. 153:375)

B. Work Achieved by November 1962.

By mid-november tissues had been processed from a group of 20 male albino rats of about 2 weeks of age (50 gms) and 21 male syrian golden hamsters of a similar age, in addition to 1 female adult maccus rhesus monkey.

A colony of 25 male syrian golden hamsters was established, in order to provide tissue from elderly male hamsters sometime next summer. Arrangements have been made with the department of physiology to purchase elderly male albino rats early in the new year.

The following is a résumé of the observations to date;

I. The Mucosa of the Hamster Buccal Pouch

It consists of a well keratinized stratified squamous epithelium with a stratum granulosum of one cell layer in thickness, a stratum Malpighii of two to three cell layers in width, and a basal cell layer.

The submucosa is composed of a thin layer of connective tissue and muscle. In some areas the muscle is immediately adjacent to the epithelium, with only a very narrow layer of connective tissue separating them.

1. Glycogen Distribution as seen using the P.A.S. (Hotchkiss) Stain

The stratum Malpighii showed a diffuse homogenous staining pattern, with the notable exception of the nuclei which were negative. Fine intercellular lines could be seen in the basal layer running at right angles to the basement membrane. The intensity of the stain varied from one area to another in the same section. Sections previously treated with diastase were negative.

The connective tissue and muscle of the submucosa were positive, except for clear areas here and there probably representing nuclei, blood vessels, etc. The intensity of the stain diminished considerably when the sections were previously treated with diastase.

2. Glycogen Distribution as seen using the Best Carmine Stain

The strata granulosum and Malpighii and the basal cell layer were positive, with the exception of the nuclei in stratum Malpighii were usually negative. In contrast to the P.A.S. stain, the nuclei of the basal layer were positive, while the intercellular spaces were negative.

In the submucosa the stain was limited to the nuclei in the connective tissue, the cytoplasm staining very faintly.

II. The Buccal Mucosa of the Rat

The rat buccal mucosa differs from that of the hamster in its proportions only, all the strata previously mentioned are wider in the rat.

1. Glycogen Distribution as seen using the P.A.S. (Hotchkiss) Stain

The glycogen distribution in the rat using this stain as a criteria, differs from that seen in the hamster.

It is mostly limited to the intercellular areas of the epithelium in the stratum Malpighii, and to a lesser degree in the basal layer. The distribution in the stratum Malpighii is not as diffuse as that seen in the hamster.

2. Glycogen Distribution as seen using the Best Carmine Stain

The nuclei and to a lesser extent the cytoplasm of the strata granulosum, malpighii and basal cell layer were all positive with this stain.

The intercellular spaces of the epithelium remained negative.

III. Quantitative Measurements

The amount of data accumulated to date is insufficient to make valid comparative observations at this time.

C. Future Plans

A similar analysis of the glycogen content and distribution in the buccal mucosa of senile hamsters and rats will be made. The effect of various general (diet, temperature variations) and localized (application of carcinogens) influences on the glycogen content and distribution in the mucosa will be studied.

Arrangements have been made to obtain fresh specimens of human buccal mucosa from the students in the faculty of dentistry. It is hoped that the observations made in this part of the project will serve as a basis for future comparisons.

PROGRESS REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH
1962-63

GRANTEE : C.M. Dowse University of Manitoba

PROJECT NO.: DA-81

PROJECT TITLE: Metabolism of Periosteum.

During the past year work on this project was principally devoted to the study of the metabolism of glucose- U-C^{14} by calvarial preparations as set out in detail in the following report.

METHODS

Material and incubation procedure.

Preparations of calvaria from new-born Long Evans rats were used. In previous work almost the whole calvarium comprising the frontal, parietal and upper half of the occipital bones had been used, on the assumption that the tissue was cartilage free. However, continuing histological studies indicated a small amount of cartilage usually occurred in the occipital bone and infrequently in the sagittal suture. To eliminate this the occipital bone is now removed leaving only the parietal and frontal bones. These are divided in the midline and the median aspect of the parietals also removed. As a considerable number of animals were required to provide the requisite amount of material, the skulls were collected in ice cold glucose free medium. If more than one incubation was performed the halves of the skulls were randomised among the flasks within any one experiment. Incubation was carried out in Ca free Krebs Ringer phosphate medium for 2 hours in air at 37°C . Glucose was present at a concentration of $1.11 \mu\text{Moles per ml}$. and uniformly labelled glucose- C^{14} added at the specific activity indicated in the separate experiments.

Analyses.

CO₂ was determined manometrically. Lactic acid was determined by the lactic dehydrogenase - DPN method of Horn and Bruns (1956). Glucose was determined by a glucose oxidase - 3:3' dimethoxybenzidine method modified from that of Kingsley and Getchell (1960). Separation of lactic acid from the incubation medium was accomplished by elution from 1 x 7 cm columns of Dowex 1 (Cl⁻ form, X-8, 200-400 mesh) using a linear HCl gradient, 0 - 0.2N over 400 mls, after the manner of Kinoshita, Masurat and Helfant (1955). Fractions of 4 ml. were collected.

Radioactivity determinations.

C¹⁴ was assayed as BaC¹⁴O₃ after precipitation and filtering onto 25 mm Millipore filter discs (HA or RA grade). The filter discs were held down in stainless steel planchets by brass rings and counted using a thin window gas flow tube. Cpm obtained were corrected to infinite thinness using the weight of BaCO₃ and the self-absorption correction

$$\frac{I}{I_0} = \frac{1 - e^{-.29L}}{.29L}$$

where L is weight of BaCO₃ (mg/cm²)

I is observed activity

I₀ is activity at infinite thinness.

Samples of CO₂ in NaOH were precipitated directly as BaCO₃. Lactate and glucose were combusted to CO₂ by a modification of the potassium persulphate method described by Abraham and Hassid (1957). In all cases enough inactive carrier was added before precipitation or oxidation respectively to yield about 10 mg BaCO₃. This gave a plating density of about 2 mg/cm² which is the optimal point on the BaCO₃ self-absorption curve.

EXPERIMENTSExperiment #1

Approximately 100 mg. of bone was placed in 1.5 mls of medium in each of six Warburg flasks. Glucose-U-C¹⁴ (1.96×10^4 cpm/ μ M) was present in the medium in four which were incubated for 2 hours, the two control flasks contained no glucose. Incubation was stopped, at time zero in the control flasks and after 2 hours in the experimental flasks by the addition of 250 μ l of 17.5% trichloroacetic acid from the side arms. CO₂ evolved was absorbed in 250 μ l of 2N NaOH in the centre well. Recording of the O₂ uptake during incubation enabled the Q_{O₂} to be determined. The NaOH from the centre wells was transferred by suction to 5 ml. volumetric flasks, made up to volume, and suitable aliquots removed for determination of the CO₂ content. In this way the metabolic CO₂ production could be estimated. Aliquots were also removed for activity determinations.

The protein free trichloroacetic extracts were neutralized with KOH and 100 μ l aliquots removed for determination of the lactic acid content. This was zero in the control flasks, i.e. there was no lactic acid present in the tissue at the beginning of incubation. After addition of 5 mg. of carrier lactic acid the remainder of the neutralized extracts from the experimental flasks were placed on columns of Dowex 1 which were eluted as described under Methods. The eluant fractions containing the lactic acid were identified by chemical analysis and the apparent specific activity of the lactic acid calculated after oxidation of suitable aliquots. It was noticed that over the eight or so fractions containing lactic acid the apparent specific activity was uniform for the last four. This value was taken to be the true specific activity. In the earlier fractions the apparent specific activity was considerably higher indicating incomplete separation from another labelled compound which was later (cf. Experiment 2) shown to be glutamic acid.

Experiment #2

Two 150 ml Erlenmeyer flasks with fused-in centre wells containing NaOH were used for incubation of larger amounts of tissue than in Experiment #1. Incubation conditions were similar to those in Experiment #1 except that the specific activity of the glucose-U-C¹⁴ (1.30×10^5 cpm/ μ M) was about 8 times higher. Trichloroacetic acid was added by syringe through a serum stopper inserted in the stopper of the flask. The CO₂ collected in the centre well was assayed as before, lactic acid and glucose determinations were performed on the neutralized protein free trichloroacetic acid extracts and lactic acid separated on Dowex 1 without the addition of carrier lactate. The same variation in apparent specific activity was observed. The fractions containing lactic acid were freeze dried and aliquots chromatographed on Whatman #1 using the buffered phenol solvent A of McFarren (1951). Glutamic acid was identified in the earlier fractions and its specific activity determined.

Experiment #3

This was carried out with two main objects in mind, to obtain further information on the presence of label in intermediates such as glutamic acid and to determine in a preliminary way if the specific activity of the metabolic CO₂ could be assessed more accurately by collecting and assaying only the CO₂ produced during the incubation rather than the total after addition of acid. The bone was incubated in a flask as in Experiment #2 with glucose-U-C¹⁴ present at a specific activity of 1.30×10^6 cpm/ μ M. After 2 hours the tissue was removed, rapidly washed with water and extracted for 1 hour periods successively with boiling 80% ethanol, boiling 20% ethanol and boiling water. The extracts were pooled, freeze dried and aliquots subjected to two dimensional chromatography on oxalic acid washed Whatman #4 paper with phenol-H₂O and butanol-propionic carboxylic acid-H₂O as solvents. Standard mixtures of amino acids and/acid were

run similarly. The experimental sheets were exposed to Ilfex No-Screen X ray film for periods of 5 - 20 days.

After extraction as above the tissue remaining was decalcified by dialysis against EDTA, then washed by dialysis against water. The material remaining was dried on a planchet weighed and counted.

The lactic acid content of the medium and its specific activity after separation was determined as before. The amount and specific activity of the CO_2 collected in the centre well was determined as previously described.

RESULTS AND DISCUSSION

The numerical results at present available and certain calculations from them to be used in the following discussion are given in detail in Table 1.

Recovery of C^{14}

Only 36% of the glucose- U-C^{14} metabolised was recovered in Experiment #2 in the intermediates isolated. About 28% was present in the lactic acid formed, 7% in the CO_2 and 1% in the glutamic acid. Similar results were obtained in Experiments #1 and #3 as to the fraction of activity originally present in the medium which appeared in the lactic acid and CO_2 . Assuming the rate of glucose uptake was similar in all experiments, therefore, about $2/3$ of the label metabolised has

not yet been accounted for. The results of Experiment #3 which are still incomplete indicate the fate of a part of this. From the radioautograms of the two-dimensional chromatograms of the tissue extract, label was presently in approximately equal amount in alanine, aspartic acid and glutamic acid, but not apparently in glycine. Lactic acid, of course, was heavily labelled and faint spots corresponding to some of the phosphorylated glycolytic intermediates were present. No trace of activity could be observed in the positions which would be occupied by any of the Krebs cycle intermediates, nor could these be visualized chemically. However, in view of the fact that many of these acids combine with bone mineral, their concentration in an alcohol:H₂O extract may be very low. Cohn and Forscher (1962a) found less than 1% of the utilized activity present in Krebs cycle intermediates separated on silic acid from a trichloroacetic acid extract of metaphyseal preparations incubated with glucose-U-C¹⁴ under conditions similar to the present ones. They could recover about 60% of the activity in lactic acid and about 5% in the CO₂. The residual tissue in Experiment #3 after extraction and decalcification counted at 1×10^4 cpm when directly plated. It is reasonable to assume that this material is principally protein, and it is intended to hydrolyse it, separate the amino acids, determine their specific activity and compare this with that of the acids present in the free form in the alcohol extract.

Lactic acid production and specific activity

Although the production of lactic acid per gm. of tissue was quite variable an inverse correlation between this and the specific activity of the lactic acid was present so that the fraction either of activity utilized or of total original activity which was present in the lactic acid formed was much more constant. This is especially apparent in Experiment #2 where a two fold difference existed in the net lactic acid production yet in both flasks a very similar fraction, approximately 28% of the activity utilized, was present in the lactic acid. The ratio of the specific activities of glucose and lactic acid ranged from 2:1 to 10:1 indicating a dilution of the glucose to lactic acid pathway by an endogenous substrate. It has been shown earlier that glycogen present in the tissue can produce considerable quantities of lactic acid under conditions where no external substrate is added. Because of the marked variation in lactic acid production noted above, yet the apparent constancy of the glucose uptake it is argued that the concentration of glucose selected has been such as to allow varying degrees of dilution by endogenous substrate. This it is thought is probably glycogen. Cohn and Forscher (1962a) found the specific activity of the lactic acid produced by metaphyseal preparations incubated with glucose- $U-C^{14}$ in bicarbonate buffer to be the same as that of the glucose. The glucose concentration they used was initially higher than that in the present study, 1.20 as compared to 1.11 μ moles/ml. However, no data is available directly concerning the glycogen content of their preparations, though Laskin and Engel (1956) demonstrated glycogen in similar preparations from adult rabbits. The different findings of Cohn and Forscher were at first thought, therefore, to be due either to the use of a higher initial glucose concentration or the absence of a similar endogenous substrate in their preparations. In their latest paper however, (Cohn and Forscher 1962b), are

reported results which show that in the metaphyseal preparation they utilize the equivalence in moles of lactic acid produced to glucose consumed is not as constant as appeared to be the case from their earlier work. From figures given in Table 1 of their paper (unfortunately means of 8 or 9 incubation flasks with no measure of the variance) it can be calculated that the equivalence in moles of glucose consumed to lactic acid produced varied from 1:0.9 to 1:1.5. They do not report the specific activity of the lactic acid though it was apparently measured so no final conclusion as to the constancy of this parameter in their system can be drawn.

Metabolic CO₂ production

The error inherent in attempting to determine by difference what amounts to approximately a 10% change in the CO₂ content of the system places a limit on the reliability of the estimation of this parameter carried out in Experiment #1. It has been found, however, that although values obtained for the CO₂ content of calvarial preparations may differ from day to day, quite uniform results are obtained for replicate samples from a single randomised collection of calvaria on any one day. The average value for three flasks in Experiment #1 was 16.7 $\mu\text{M/gm.}/2$ hours which when compared to the Q_{O₂} determined of 16 $\mu\text{M/gm.}/\text{hour}$ indicated an R.Q. of about 0.5. Because of the labile nature of the CO₂ in such bone preparations demonstrated in Experiment #3 and in metaphyseal bone preparations as shown by Borle et al (1960) it is almost certain that attempts to determine the Q_{CO₂} and Q_{O₂} by differential manometry will be unsatisfactory. Because of the somewhat low value for the RQ obtained further study is necessary of the reliability of this method for determining metabolic CO₂ production.

Metabolic CO₂ specific activity

The specific activity of the estimated metabolic CO₂ was considerably less than that of the lactic acid, being of the order of 1/5 - 1/10 that of

the latter, and only $1/20 - 1/40$ that of glucose. At least three tentative conclusions may be drawn from this fact. First that a large part of the CO_2 is produced elsewhere than in the HMP pathway. Second that a second endogenous source of substrate exists in the tissue after pyruvate and third that a major fraction of the metabolic CO_2 arose from sources other than the glucose added. This finding of a very low specific activity of CO_2 relative to that of glucose makes it incorrect to conclude as did Cohn and Forscher (1962a) that the TCA cycle is of little consequence in bone on the basis of the fraction of activity from labelled glucose which is incorporated into CO_2 . It also makes the ratio of activity of CO_2 produced from glucose labelled at carbon atoms 1 and 6 an unreliable guide to the pathways of glucose metabolism.

CO_2 production and specific activity in Experiment #3

A surprisingly large amount of CO_2 was evolved during the incubation in this experiment, amounting to some 50% of the total CO_2 estimated to be present in the tissue. This is evidence of the markedly labile nature of the CO_2 present in such preparations of bone. It is interesting to note that Borle and Nichols (1960) produced evidence for the reverse phenomenon, i.e., a considerable net uptake of CO_2 during incubation in bicarbonate medium of metaphyseal preparations from mice. In a later paper, Vaes and Nichols (1962), they assume, somewhat surprisingly, that exchange of metabolically produced CO_2 with the carbonate in the mineral of similar preparations will be minimal on the basis of earlier work by Buchanan and Nakao (1952). However the studies of Buchanan and Nakao were concerned with in vivo turnover rates of CO_2 in whole bones and probably have little relevance to the in vitro conditions of the present work and that of Nichols et al. The total activity present in the CO_2 collected in Experiment #3 was equivalent to 2.2% of the original activity, present as glucose- U-C^{14} , only

slightly lower than the corresponding value obtained in Experiment #2. This suggests that although a large net movement of CO_2 out of the tissue had occurred equilibration of specific activity throughout the CO_2 present in the system was incomplete.

CONCLUSION

Before experiments designed to elucidate the intimate quantitative details of the metabolism of a particular preparation of bone tissue and to pinpoint the action of parathyroid hormone can be performed it is obvious that more knowledge of a general nature must be obtained. It is clearly unsound, as pointed out in detail by Katz and Wood (1960) to utilize the ratio of label appearing in any intermediate from variously labelled glucose as an assessment of the relative amount of metabolism occurring by different pathways unless certain criteria are met. Of immediate concern in the present situation are (i) the fact that endogenous dilution occurs to a varying degree and (ii) that the presence of only some 30 - 35% of the label from glucose- U-C^{14} in the lactate and CO_2 makes the metabolism of a major fraction of the glucose by non-triose P pathways, specifically into glycogen, a possibility that cannot be excluded. Until the extent and variability of the endogenous dilution and the fraction of glucose being metabolised other than by the HMP and Embden-Meyerhof pathways can be assessed and controlled no useful data can be obtained using variously labelled substrates. The ways in which these and other problems raised by the work of the past year will be approached are outlined in the present application.

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TABLE 1

SAMPLE #	EXPERIMENT #1				EXPERIMENT #2		EXPERIMENT #3
	1	4	9	7	1	2	1
Wet Weight of Tissue (mg)	107	109	105	100	789	589	270
Volume of Medium (ml)	1.5	1.5	1.5	1.5	10	7.5	3.75
Glucose Initial (μM)	1.67	1.67	1.67	1.67	11.1	8.33	4.16
Glucose Utilized (μM)	1				4.21	3.19	
Utilization rate ($\mu\text{M}/\text{gm.wet}/2 \text{ hrs}$)					5.33	5.33	
S.a. Glucose (cpm/ μM)	1.96×10^4	1.96×10^4	1.96×10^4	1.96×10^4	1.30×10^5	1.30×10^5	1.30×10^6
Total Counts Original (cpm)	3.34×10^4	3.34×10^4	3.34×10^4	3.34×10^4	1.44×10^6	1.08×10^6	5.41×10^6
Total Counts Utilized (cpm)					5.48×10^5	4.15×10^5	
Lactic Acid Produced (μM)	1.42	0.95	1.17	1.09	6.20	2.42	2.30
Production Rate ($\mu\text{M}/\text{gm}/2 \text{ hrs}$)	13.2	8.7	11.1	10.8	7.90	4.11	8.5
S.a. Lactate (cpm/ μM)	1.61×10^3	3.44×10^3	2.13×10^3	2.85×10^3	2.61×10^4	4.68×10^4	2.25×10^5
Total Counts in Lactate (cpm)	2.28×10^3	3.26×10^3	2.49×10^3	3.08×10^3	1.62×10^5	1.13×10^5	5.18×10^5
% of Total Counts Utilized (%)					29.6	27.2	
% of Total Counts Original (%)	6.8	9.8	7.5	9.2	11.2	10.5	9.6
CO ₂ Total Found (μM)	25.3(1)	21.0	19.4	19.3	130.0	90.9	24.5(3)
CO ₂ Total Found ($\mu\text{M}/\text{gm}$)	236	192	185	193	164.7	154.5	90.7

TABLE 1 (continued)

S.a. CO ₂ Total (cpm/ μ M)	33.0	44.9	56.3	55.0	2.81 x 10 ²	2.99 x 10 ²	4.93 x 10 ³
Total Counts in CO ₂ (cpm)	8.3 x 10 ²	9.4 x 10 ²	10.9 x 10 ²	10.6 x 10 ²	3.65 x 10 ⁴	2.73 x 10 ⁴	1.21 x 10 ⁵
% of Total Counts Utilized (%)					6.7	6.6	
% of Total Counts Original (%)	2.5	2.8	3.3	3.2	2.5	2.5	2.2
Metabolic CO ₂ Production (μ M/gm/2hrs)		19.3	11.6	19.3	16.7 ⁽²⁾		
Metabolic CO ₂ Production Total (μ M) in 2 hrs		2.1	1.2	1.9	13.2	9.8	
S.a. Metabolic CO ₂ (cpm/ μ M)		4.5 x 10 ²	9.0 x 10 ²	5.6 x 10 ²	2.76 x 10 ³	2.90 x 10 ³	
Glutamate Total Found (μ M)					1.19		
S.a. Glutamic Acid (cpm/ μ M)					3.75 x 10 ³		
Total Counts in Glutamic (cpm)					4.4 x 10 ³		
% of Total Counts Utilized (%)					0.8		

(1) Contaminated with atmospheric CO₂ during transfer, therefore not used in estimation of metabolic CO₂.

(2) Average value estimated from results of Experiment #1.

(3) No acid added in Experiment #3. This figure represents CO₂ evolved from tissue and medium during the 2 hour incubation only.

APPENDIX F

EFFECTS OF DRUG ADMINISTRATION ON GINGIVAL HISTAMINE AND SEROTONIN.

INTRODUCTION

L.E. Francis - DT-76

Since the discovery of the anti-epileptic action of diphenylhydantoin, it has been repeatedly observed that some patients undergoing treatment develop gingival changes. These changes appear superficially like a low grade inflammatory reaction, showing irregularity of the gingival surface, but progressing slowly to a firm, painless overgrowth of the gums. Histological examination of sections of the affected gums have revealed a proliferation of capillaries and connective tissue associated with a thickening of the epithelial surface, with marked increase in cell numbers or hyperplasia. It has also been observed that stopping the administration of the drug leads slowly to restitution to normal. The hyperplasia appears therefore to be of a benign or non-malignant type. There is no specific therapy for this condition. Good dental care appears to help but does not prevent it, and in cases of excessive hyperplasia, surgical excision by the dentist has often been resorted to. This, however, only affords temporary relief and with continued therapy remission ensues.

The drug is employed in the form of diphenylhydantoin sodium, which is chemically, 5,5-diphenylhydantoin sodium, or 5,5-diphenyl 2,4-imidazolidinedione sodium, or 5,5-diphenyl hydantoinate or phenytoin sodium. It is also known under various proprietary names as Dilantin, Dilantin sodium, Dihydan soluble,

Alepsin, Epanutin, Eptoin, Hydantal, Solantoin, Solantyl and Zentropil.

Despite the many theories which have been advanced during the past 20 years concerning the possible mechanism of production of gingival hyperplasia associated with diphenylhydantoin therapy in man, there has been surprisingly little experimental investigation of this problem. Undoubtedly, one of the main reasons for this was the early report, that even following prolonged administration of the drug to experimental animals, no "gingival changes" ensued. However, the possibility arises that in many of the earlier studies in this field no particular attention was directed to the gingiva. Indeed, in Drill's Pharmacology in Medicine, Second Edition, 1958, it is stated that "prolonged administration of anti-convulsant doses of the drug (diphenylhydantoin) in animals, fails to produce pathologic changes in the tissues". While this is still current opinion, it should be stated that in 1952, King reported that following prolonged administration of the drug to ferrets, both gross and microscopic changes resembling those occurring in man, could be observed. These workers however, suggested that this occurred only under special dietary and other conditions.

In view of the above it appeared worthwhile to reinvestigate the effects of administration of diphenylhydantoin upon tissue changes in various laboratory animals, with special reference to those occurring in gingival tissue. It was soon apparent that in certain instances, despite the absence of any observable gross changes, varying degrees of histological alterations could be

detected in the gums and lungs of some of the experimental animals. This suggested that diphenylhydantoin might induce local chemical changes in some tissues, especially gingiva, and that such changes might act as "trigger mechanisms" leading progressively to microscopic and later characteristic macroscopic changes.

Since the lungs, skin and mucous membrane are rich in histamine, on the basis of the above hypothesis, it was decided to undertake a comparative study of the "histamine content" of normal gingiva and the gingiva obtained from diphenylhydantoin-treated animals. Although the exact role of histamine in tissues is still obscure, evidence exists that it plays some part in anaphylactic and allergic responses. Although it has been repeatedly suggested that the gingival changes associated with dilantin therapy in man, might be of an allergic nature, there have been no previous experimental studies in the literature concerning histamine changes in the gingiva in this condition.

It is now well established that histamine is found in mast cells, and Riley and West (1953) have shown that there appears to be a close parallel between the histamine, heparin, and mast cell contents of several tissues, although there have been no systematic studies on this question in respect to gingiva. The possible physiological and pathological roles of histamine in the gingiva appear therefore to warrant investigation.

Another important tissue substance which has recently been identified and which occurs in mast cells is 5-hydroxytryptamine or serotonin. In conjunction with this study it was

therefore of interest to compare the "serotonin content" of normal gingival tissue and that obtained from diphenylhydantoin-treated animals. There are again no previous studies in the literature on this question.

RESULTS OF EXPERIMENTS

In summary, the experiments showed the following:-

- 1 - Repeated oral administrations of diphenylhydantoin (Dilantin) to albino rats, hooded rats, guinea pigs, hamsters and weanling rats for 3 months in relatively high non-toxic doses, lead to no gross gingival changes. Histological examination of the rat's gingiva showed however, occasional mild hyperplastic changes.
- 2 - Repeated local injections of a dilantin suspension (0.2%) into the muco-buccal fold of rats lead to no gross gingival changes nor marked local visible inflammatory reactions. Microscopically, the gingiva showed varying degrees of hyperplastic changes, but skin, kidney and spleen appeared to be quite normal.
- 3 - Repeated daily oral administrations of dilantin to growing young and adult ferrets for periods ranging from 20 to 60 weeks, in doses of 20 to 200 mg., lead to gross and microscopic gingival hyperplasia of varying degrees. Some microscopic hyperplastic changes were also seen in the lungs, but none of the other tissues examined (skin, liver, spleen and kidney) showed any abnormal changes. The intensity of the gingival changes did not appear to depend upon the dosage, but required at least 12 weeks before any gross changes in growth were detectable.

4 - In all of the growing young ferrets, the dentition erupted in poor alignment, in association with the extreme hyperplasia.

5 - Extracts obtained from gingival tissue of ferrets, both normal and dilantin-fed, contain histamine, or some histamine-like substance, and the mean concentration is significantly higher in the dilantin-fed group, as judged by assays on the guinea pig ileum.

6 - Following "test doses" of extracts obtained from normal ferrets, the responses of the guinea pig ileum to histamine are temporarily diminished, but conversely, following extracts from dilantin-fed animals, the histamine responses are potentiated.

7 - Following repeated oral daily administration of dilantin to dogs for periods ranging from 7 to 63 days, in doses ranging from 40 to 200 mg., no gross gingival changes developed. Some preliminary microscopic studies of gingiva excised after 42 days, however, showed some hyperplasia.

8 - Extracts of gingival tissue prepared from both normal and dilantin-fed dogs contain histamine in variable amounts, but the mean concentration is definitely higher in the dilantin-fed animals.

9 - Following repeated "test doses" of extracts from normal dogs, the responses of the guinea pig ileum to the extract diminish strikingly and the effects of standard histamine are also temporarily diminished or prevented. Conversely, with repeated 'test doses' of extracts from dilantin-fed dogs, the subsequent responses of the ileum to the extract itself or to histamine, are either unchanged or potentiated.

- 10 - Following prolonged administration of mesantoin or phenobarbital sodium to dogs, in doses of 100 mg. per day, similarly prepared extracts show identical responses to those observed with extracts from the normal untreated animals.
- 11 - Extracts of normal gingiva of the dog contain relatively high quantities of extractable 5-hydroxytryptamine and following prolonged dilantin administration no evidence of 5-hydroxytryptamine is detectable in the extracts, using two assay methods.
- 12 - Extracts prepared from normal, inflamed and hyperplastic human gingival tissue showed that normal gingiva contained approximately 40 µg. of histamine per gram of gingiva. While inflamed gingival tissue showed 60 - 100 µg. of histamine per gram of tissue, and that hyperplastic gingival tissue was similar to inflamed tissue. However, it was noted that in normal human gingiva a histamine antagonist was also extracted which inhibited the extracted histamine on the gut. The inflamed and hyperplastic tissue showed no or little evidence of the antagonist.

CONCLUSIONS

It is concluded that the gingival hyperplasia induced in ferrets following diphenylhydantoin show considerable gross and histological similarities to that observed in man; that the condition appears to be an 'allergic' or 'hypersensitization' effect induced by the drug on the gingiva, although some histological evidence of hyperplastic changes has also been detected in the lung; that histamine and 5-hydroxytryptamine appear to be

of considerable physiological importance in gingival function, and characteristic changes in the concentrations of these, can be detected in both ferrets and dogs fed diphenylhydantoin; that mesantoin and phenobarbital sodium do not appear to lead to any abnormal gingival changes nor changes in histamine or serotonin and that the histamine content of inflamed human gingiva is higher than that in lung.

It is postulated that the above-described gingival changes are not direct effects of the drug, but rather result from secondary hypothetical "trigger mechanisms" concerned with mitosis, and probably involving alterations in histamine and 5-hydroxytryptamine. Since the histamine inhibitor exists in tissue it might be conceivable to think in terms of it being a stabilizer for "nascent histamine". Because of its absence in hyperplastic tissue the possibility of free histamine being the "trigger mechanism" for growth was considered. On the basis of this assumption a study of this substance has been started.

ANTI-HISTAMINE ISOLATION

The inhibitory substance appeared at first to exist in normal gingiva only, and appeared to be absent in chronically inflamed and in hyperplastic gingival tissue produced by dilantin. However, with more refined methods for the extraction of the inhibitor, it was seen that it was present in inflamed, cancerous and hyperplastic tissue as well as most normal tissues, but in varying amounts. Since human tissue (particularly normal) is so

difficult to acquire in adequate amounts, the dog, both normal and dilantin-fed, was also investigated.

EXTRACTION PROCEDURES

All tissues were first examined histologically and weighed, extracted and assayed for histamine activity using the method of Barsoun and Gaddum (1935). The assays were done on strips of guinea pig ileum (approximately one inch in length), placed into a 4 ml. bath containing atropinized tyrode solution suitably aerated. Following the histamine bioassay, the histamine inhibitor was then separated from the histamine. Using the following method the resultant substance was re-assayed:-

The tissue was chopped in small pieces and boiled under reflux with 1000 to 1500 ml. concentrated hydrochloric acid for two hours. The cooled mixture was filtered by suction and the filtrate was extracted twice with petroleum ether to remove fatty acids. The aqueous was saturated with ammonium sulphate, and the solids which precipitated were removed by filtration. The filtrate was then extracted with four portions of ethyl acetate, and the ethylacetate washings were combined, dried with anhydrous sodium sulphate and evaporated at 40°C in vacuo. The resulting product was extracted with four portions of carbon tetrachloride in the presence of anhydrous sodium sulphate. The carbon tetrachloride extract was evaporated in vacuo, yielding a brown oil. Acidic impurities were removed from the product by

neutralization with potassium carbonate and subsequent extraction of the inhibitor with ether.

Further purification and characterization was carried out by chromatography on paper in butanol acetic acid H_2O 5:1:4. The inhibitor was located in the region of R_f 0.83 to 1 by cutting serial sections from the chromatogram, eluting with aqueous ethanol and testing the eluates after evaporation, and bioassay. The component so identified exhibited histamine and 5-HT inhibitory activity.

RESULTS

Human Normal Gingival Tissue.

1. Antihistamine of normal gingival tissue - shows complete block.
 2.8 mg. shows complete block and 50% return in 44 mins.
 0.5 mg. " " " " " 64 mins.
2. Inflamed gingival tissue showing no depression with 190 mg. of tissue.
3. Early hyperplasia tissue shows complete block
 1065 mg. shows complete block and 50% return in 20 mins.

Human Colon Tissue.

4. Normal colon
 80.4 mg. shows complete block and 50% return in 96 mins.
5. Cancerous colon (Tumor)
 226.8 mg. shows complete block and 50% return in 40 mins.

Dog Tissue Gingiva and Brain

6. Normal dog tissue gingiva and brain
 gingiva-42.4 mg. caused depression and 50% return in 58 mins.

brain-320 mg. caused depression and 50% return in 90 mins.

7. Dog on Dilantin gingiva and brain

gingiva-18 mg. and 36 mg. caused no depression

brain-640 mg. caused depression and 50% return in 31 mins.

gingiva-480 mg. caused 5-HT depression and 50% return in 31 mins.

SUMMARY

1. It has been shown that human and dog tissues contain a histamine and 5-HT inhibitor.
2. That gingival tissue (particularly human) is very rich in this inhibitor.
3. That tissues from diphenylhydantoin-treated humans and dogs exhibit less of the inhibitor.
4. That inflamed and cancerous tissues contain less of the inhibitor than normal tissues.

8. Experimentation to the anterior chamber of the eye.

35-day old hamsters had 3rd molars removed and explanted heterologously to the guinea pig and rabbit eye. The teeth were recovered after 7 days with no clinical evidence of disease development. Histologic study revealed massive inflammatory cell invasion into the pulp chamber resulting in necrosis of pulp tissues. Absorption of calcified structures rather than deposition was evident on microscopic examination. This classic

ABSTRACT OF PROGRESS REPORT - N.R.C. 281-25

Studies on Tooth Transplantation

L.E. Francis DA-88

I. The Development of an "In Vivo" Site for Third Molar
Explantation to be Used in Further Evaluation of
Tissue Culture Procedures.

A. Explantation to the axillary space.

Developing hamster 3rd molars were extracted and planted autogenously in the axillary space in control and cortisone treated hamsters. The teeth were recovered after 7 days and examined histologically. In control specimen the pulp chamber was filled with inflammatory cells, principally macrophages, leukocytes and giant cells. Pulp tissue was either replaced or undergoing necrosis. In cortisone modified teeth, axillary connective tissue had proliferated into the pulp, but there was a marked reduction of the inflammatory reaction. Cortisone was seen to have reduced the cellular response but this did not alter the fate of the explanted pulp.

B. Explantations to the anterior chamber of the eye.

35-day old hamsters had 3rd molars removed and explanted heterologously to the guinea pig and rabbit eye. The teeth were recovered after 7 days with no clinical evidence of continued development. Histologic study revealed massive inflammatory cell invasion into the pulp chamber resulting in necrosis of pulp tissues. Resorption of calcified structures rather than deposition was evident on microscopic examination. This classic-

al cell-free, non-antigenic graft site was seen to be grossly insulted by the presence of calcified tissue and a violent foreign body reaction was elicited.

C. Peritoneal implantation of Millipore diffusion chambers.

Hamster third molars were introduced into a partially assembled 2-lucite ring diffusion chamber; the rings were closed and sealed. Decontamination in a sterilization bath was undertaken prior to insertion into the peritoneal cavity of the donor animal. The operative and post-operative course was uneventful. Recovery was complicated by visible inflammation on the 8th and 16th day. Histologic examination revealed excellent preservation of dental tissues including odontoblasts within the first 2 days. The 4-day and 8-day specimen showed progressive cellular necrosis in the apical pulp while 16-day recoveries had similar changes in the deeper tissues. However, even in the 16-day explants, odontoblasts and pulp elements in the pulp horns showed only minimal necrosis, i.e. moderate granulation and swelling of cytoplasm with good nuclear detail and staining properties.

The relation of clinical suppuration in the later recoveries and the pattern of cellular necrosis suggests diffusion of toxins from the external inflammation into the pulp tissue. The chronology of the necrosis in the peripheral tissue fragments, the periodontal membrane and the epithelial attachment support this interpretation. No inflammatory cells had entered the pulp chamber or periodontal tissues. These findings suggest that elimination of the external inflammation presently being attempted

with procedural and technical modifications will establish the Millipore filter chamber as a site for growth and development of tooth explants.

II. Studies on the Development of a Medium for the "In Vitro"
Storage of Potential Tooth Transplants.

A test system was designed to facilitate evaluation of various culture media. Free pulp was obtained by fracturing hamster third molars under the dissecting microscope, enucleating the pulp and dividing it into fragments. These were passed through an antibiotic bath and planted on the cover slip of an Yerganian culture tube. Basic culture media was then added and the tubes incubated at 37°C. Gross and microscopic examinations were done during the culturing phase. On recovery, the small fragments were orientated on Millipore filter and sectioned.

Initial studies with this method focussed on basic Eagle's and Hanks' balanced salt solutions with saline controls. Tissue was recovered following 1, 4 and 16 days of storage. Clinical observations did not suggest proliferation.

Histological assessment resulted in the following conclusions:-

- (1) First day recoveries from the three media were generally identical with uncultured H. and E. stained pulp tissue.

- (2) The 4-day grafts cultured in Eagle's and Hanks' were similar and characterized by central necrosis with broad margins of normal odontoblasts and pulp elements. Saline controls were necrotic.
- (3) All 16-day specimen showed cellular necrosis. Those cultured in Eagle's had peripheral islands of relatively normal pulp tissue; the saline and Hanks' were necrotic throughout.

The test system proved entirely satisfactory as a simple culture method. The limiting factors in preservation seemed to be duration and diffusion. Studies are proposed to assess media designed to improve the nutrient capacities, or to increase diffusion, or to reduce the "in vitro" metabolic requirements of the tissue.

PROGRESS REPORT ON N.R.C. 281-25"STUDIES ON TOOTH TRANSPLANTATION"

I. Development of an "In Vivo" Site for Third Molar
Explantation to be Used in Further Evaluation of
Tissue Culture Procedures.

Previous studies on the storage of potential tooth transplants indicated the need for a more definite procedure for evaluating "in vitro" preservation of dental tissues than oral transplantation. Thus the first phase of N.R.C. 281-25 was designed to explore an "in vivo" site for third molar explantation which would allow continued growth and development. Studies were again undertaken in the golden hamster because of the similarity of this rodent's molars to the human.

A. Explantation to the axillary space.

Thirty-five day-old hybrid golden hamsters were obtained from Bio-Research Laboratories, Boston, Massachusetts. All animals had lower right and left third molars extracted and introduced with French trocars into the axillary space through a single mid-line incision. Trauma and contamination were minimal. Ten animals received 3 mg. of cortisone acetate (Cortone, Merck) I.M. immediately and 1 mg. every third day until recovery on post explantation day 7.

Clinically little difference could be seen in the two groups. The animals developed no gross signs of toxicity to the cortisone, nor signs of inflammation associated with the grafts.

A representative control tooth was characterized by

(1) replacement of the pulp tissue by a dense fibrous connective tissue with absence of any normal pupal elements;

(2) deposition of cellular osteodentin at the apex of the root;

(3) foreign body reaction surrounding the specimen and entering the pulp canal for varying depths. The cellular elements were mainly lymphocytes, macrophages and occasional giant cells. The quantity of the reaction varied with different specimen but was absent in none. Specimen recovered from cortisone modified animals showed (1) connective tissue replacement of pulp chamber and canals with loss of normal tissues. (2) Apical deposition of cellular osteodentin occasionally forming a bulb of calcified tissue on the root apex. Calcification thus continued briefly after explantation but appeared to lack orientation. (3) Reduction of the inflammatory reaction in varying degrees. Some specimen showed no round cell invasion, others had a moderate number of cells surrounding the enameled portions of the graft. In general, however, the cortisone was effective in reducing the cellular elements of the foreign body response.

In summary, the favourable decrease in the inflammatory reaction in cortisone treated animals did not significantly improve the regenerative capabilities of the pulp tissue. Specimen taken prior to 7 days suggested that pulp necrosis began within 24 hours of explantation, and that replacement from the axillary connective tissue began between 48 and 72 hours. This would suggest lack of revascularization or diffusion

to the pulp under the conditions of this study.

B. Explantation to the anterior chamber of the guinea pig.

Thirty-five day-old golden hamsters had the lower left third molar removed and placed in a sterilization culture bath to await preparation of the guinea pig eye. Adult guinea pigs, at least 6 months of age, were restrained on an operating board. The left eye was anesthetized with 5% cocaine and a 3 mm. incision was made at the superior pole of the cornea near the junction of the cornea and sclera with straight double-edged corneal knife. The tooth was removed from the bath by introduction into a French trocar and was seated via the incision at the lower pole of the eye. The animal was freed and exhibited no adverse effects.

The guinea pigs were followed for 7 days. Clinical findings were (1) the anterior chamber was generally too small for the 35 day molars. (2) Opacities suggesting inflammation, persisted in about 1/3 of the cases. (3) The best graft showed no inflammation, no protrusion through the wound, and was easily visible through the layers of the cornea. (4) No additional root development.

The animals were sacrificed after 7 days, the eyes were enucleated and the grafts dissected free. In the grossly inflamed specimen, the aqueous humor was coagulated and the grafts appeared partially resorbed. Histologic examination revealed (1) all grafts were surrounded by a massive foreign body reaction which extended deep into the pulp tissue. (2) No normal pulp

tissue remained. (3) No evidence of dentin or osteodentin deposition followed explantation. (4) Resorption of calcified tissue marked those grafts showing excessive inflammation clinically.

A few similar grafts were attempted in rabbit anterior chambers where the volume was more than adequate for the tissue. The foreign body reaction persisted with the calcified teeth. The findings were similar to those observed in axillary grafts unmodified by cortisone. The degree of the reaction was felt to be more violent in the eye chamber. Perhaps a component of the response in the guinea pig was due to an immunologic phenomenon, however, the short duration of stimulus and the supposed freedom of this site from rejection negate this interpretation. No effort was made to clarify this facet as the results of the Millipore chambers were more fruitful as regards the development of an "in vivo" grafting site.

C. Explantation of diffusion chambers containing third molars to the peritoneum of the hamster.

A group of hybrid golden hamsters, age 32 days, was obtained. Prior to operation, the animal received 5 mg. of tetracycline I.M., 1 mg. of atropine sulphate and the abdomen was freed of hair with a dibilitory agent. The lower left third molar was extracted under ether anesthesia and placed in a sterilization bath (Eagle's B.S.S. with streptomycin 0.5 mg./ml. and penicillin 1000 units/ml.) for five minutes. The Millipore chambers were previously prepared to the extent of cementing the 0.45 micron filter paper to

the two lucite rings. The filter of the larger ring was moistened with Eagle's B.S.S. and the tooth was seated on the bubble of culture medium. The smaller ring was then fitted into the larger after the method of Algire (J.Nat. Ca.Inst., 15:493, 1954). The special M.F. lucite bonding medium was used to seal the rings and the margin was evaluated under the dissecting microscope. Sterile instruments were used throughout the assembly plus masks, gloves, and drapings. Completed chambers were placed in the sterilization bath for an additional five minutes while the hamster was re-anesthetized. A left abdominal incision was made into the peritoneum and the chamber inserted with forceps to the far right. The incision was closed with 5 (0) silk and the animal was allowed to recover. The post-operative routine was normal diet, separate caging, and 5 mg. of tetracycline every 24 hours for three days. The animals were sacrificed on days 1, 2, 4, 8, and 16. Clinical findings were limited to evaluation of the operative procedure. Twenty animals were grafted in order to have at least three specimen per day. Wound healing was uneventful in all instances, three animals died late in the study from abdominal inflammation.

The chambers recovered on days 1, 2, and 4, were surrounded by a thin connective tissue capsule without apparent inflammation. Day 8 and 16 rings were generally complicated by a thick, yellowish-white pus contained in a tough bulging white capsule. The teeth had not elongated but were otherwise normal on gross examination. Histologic evaluation of the specimen showed the

following:-

(1) In the day 1 and 2 explants, the preservation of cellular detail when compared to control teeth from the right side of the same animal, was excellent. The odontoblast cytoplasm was well stained with no granularity or vascularization, the nuclei were characteristically elongated; the cells appeared normal. The remainder of the pulp tissue was free of necrotic changes and no difference could be noted between pulp and apex.

(2) The day 4 grafts showed better preservation in the pulp horn than at the apex. Necrotic changes, i.e. loss of cellular details, pale nuclei and vacuolization, were prominent at the root-end while in the deeper portions of the chamber, pulp and odontoblasts were in good repair.

(3) The day 8 and 16 teeth had necrotic pulps, but mainly in the apical third. The pathogen appeared to be advancing into the chamber with time. A middle zone of early cellular damage, a chamber with relatively healthy pulp tissue and apical necrosis characterised the day 16 explants.

(4) Remnants of the periodontal membrane became progressively necrotic similar to the pulp tissue, but the changes were accelerated.

Along with the above study, six additional hamsters received chambers containing pulp fragments and buccal mucosa. This tissue was recovered on days 1, 4, and 16. The findings were similar except for a day 1 specimen in which the membrane seal was apparently inadequate and foreign tissue was found in the chamber. The mucosa of this explant showed moderate necrotic changes. The cellular breakdown in the 16 day tissue was again related to external inflammation.

Conclusions:

- (1) The Millipore chamber represents a more promising site for preservation and possible growth of tooth explants than the axilla or anterior chamber within the protocol of this study.
- (2) The time relationship between the onset of external inflammation and pulpal necrosis, plus the reversal of the usual destructive pattern observed in previous studies, suggests the presence of toxic substances diffusing into the chamber and causing cellular destruction.
- (3) The preservation of cellular detail in the day 1 and 2 specimen and in the deep pulp tissues of later grafts was most encouraging.
- (4) The capsule formation seen around the early chambers is classic for this site and should not be a limiting factor in diffusion. The gross inflammation of later recoveries is of concern. The use of metal or heat

resistant lucite which would allow autoclaving of the partially assembled chamber; the incubation of the completed chamber in the decontamination medium for a longer interval; the use of antibiotics over the duration of the study (inflammation onset related to termination of antibiotic therapy); and the modification of animals with cortisone could likely correct this complication.

II. Studies on the Development of Media for the "In Vitro" Storage of Potential Tooth Transplants.

In an effort to facilitate the evaluation of various culture media, the following method was developed. Third molars from 30-35 day hamsters were extracted and placed in a Petri dish containing Eagle's B.S.S. with antibiotics. Under the dissecting microscope, the tooth was fractured and the pulp tissue shelled out without difficulty. Histologic study of this tissue showed typical odontoblasts and pulp elements. Sections of the fractured tooth structure showed isolated areas of odontoblasts adhering to the dentin.

The pulp was then divided into thirds or quarters, and the fragments transferred to a second sterile culture bath for five minutes. Using Pasteur pipettes, the tissue was aseptically planted on the cover glass of a Yerganian culture tube. The basic culture media was added and the tubes incubated. The explants were observed both grossly and microscopically. On

recovery, the tiny fragments were placed on Millipore filter paper for orientation. The filter and tissue were fixed, embedded and sectioned together.

In the initial efforts with this procedure, Hanks' B.S.S. with vitamins, amino acids and glutamine; Eagle's solution; and isotonic saline controls were the media evaluated. The method was as described. Periodic examination over the duration of the study showed (1) no contamination of culture media; (2) no alteration of pH in the media, and (3) no visible outgrowth from the explants.

Histologic evaluation based on H + E sections revealed the following:-

- (1) The 24-hour recoveries were similar to normal pulp sections regardless of the medium.
- (2) The 4-day specimen could be divided into those stored in saline and those in the balanced salt solutions. Differences between Eagle's and the supplemented Hanks' solution were not detectible.

Saline specimen: The fragments were characterized by central areas of marked tissue necrosis. Nuclei were absent, the normal cytoplasm and collagenous fibres were replaced by a homogeneous, granular, pale eosinophilic material. The more peripheral portions of the pulp tissue had visible nuclei in various degrees of pyknosis. The cytoplasm was granular. The odontoblast layer was seen as a structureless dense basophilic mass in which fragments of

nuclear debris could be distinguished. Tissue necrosis was well advanced.

Balanced salt solution specimen: The fragments from Eagle's and Hanks' media were not entirely free of necrotic changes, but they were balanced with peripheral areas of healthy pulp and odontoblasts. Serial sections through the tissue block suggested that diffusion into or out from the deeper layers was not adequate. Pale nuclei with indistinct cytoplasm characterized these central zones whereas cellular detail at the tissue margins was free of pathological changes.

(3) The 16-day recoveries showed cellular necrosis regardless of the media.

Saline specimen: Necrosis was prominent throughout the pulp tissue. Most areas were structureless with varying amounts of nuclear debris.

Hanks' B.S.S. specimen: Pulp elements were poorly stained and cellular detail was absent in both the peripheral and deep segments. Occasional marginal areas of pulp tissue had distinguishable nuclei, but no odontoblasts were seen.

Eagle's B.S.S. specimen: The best specimen in this medium could be distinguished from the control and Hanks', but only in terms of less cellular necrosis. Pulp tissue free of pathological change was not seen after 16 days. The fragments showing the least necrosis were characterized by a central amorphous pale eosinophilic mass with nuclear remnants surrounded by variable sized areas of tissue preservation.

Odontoblasts nuclei could be distinguished in the surface layer, but the cytoplasm was granular and poorly stained. Beneath the odontoblast layer a modest zone of connective tissue preservation was evident.

In summary, the 16 days of incubation apparently extended beyond the capabilities of the pulp fragments in the media employed. In 4 day specimen, the B.S.S.'s were superior to isotonic saline but differences between Hanks' and Eagle's could not be noted. Diffusion may be the limiting factor as necrosis of deeper tissues was evident even in earlier recoveries. Studies of less duration and with varied media are planned to substantiate these conclusions and to reduce cellular changes.

The test system itself proved to be a simple technique allowing maximum sterility with moderate preparation.

3.5% OF AIR ARE OBTAINED FROM 75 TO 85% OF THE AIR IN THE AIR (14 TO 15% MOISTURE). - THE AIR AND MOISTURE WERE POOLED SEPARATELY BECAUSE OF DIFFERENT ONCOLOGICAL DEVELOPMENTAL PERIODS. SUCH WERE IN AN IMPORTANT SOURCE OF ENERGY. WITH OBTAINED FROM CHILDREN WHO WERE BOTTLE-FED WERE SEPARATED FROM THOSE WHO WERE BREAST-FED. ALL TEETH WERE DIVIDED INTO DENTIN AND ENAMEL FRACTIONS IN ORDER TO DETECT DIFFERENCES IN THE NATURE OF ENAMEL BY THE TWO TYPES. ANALYTICAL PROCEDURES ARE DISCUSSED IN DETAIL IN THE 1961 REPORT.

RESULTS

THE LARGEST SAMPLE OF TEETH CAME FROM MONTREAL, AND DATA FROM THAT AREA ARE TABULATED IN TABLE I, AND ILLUSTRATED IN

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH
OF THE NATIONAL RESEARCH COUNCIL

GRANTEE: A. M. HUNT

PROJECT: DA 82 AMOUNT AWARDED 1962-63 \$7110.00

SUBJECT: MEASUREMENT OF STRONTIUM⁹⁰ AND CARBON¹⁴ IN PRIMARY
TEETH OF CANADIAN CHILDREN.

REPORT

THE PROJECT WAS OUTLINED IN DETAIL IN THE 1960 AND 1961
PROGRESS REPORTS. OBJECTIVES OF THE STUDY ARE: (1) TO OB--
TAIN AN ACCURATE ESTIMATE OF THE DISCRIMINATION AGAINST Sr⁹⁰
AS IT PASSES FROM COWS TO MILK TO HUMANS; (2) TO STUDY THE
UPTAKE OF Sr⁹⁰ BY CALCIFIED TISSUES WHICH HAVE DIFFERENT META-
BOLIC PROPERTIES THAN BONE.

ANALYSIS OF HUMAN PRIMARY TEETH

EIGHTY-EIGHT POOLED SAMPLES OF HUMAN PRIMARY TEETH, EACH
CONTAINING 2.5 GRAMS OF ASH, WERE MONITORED FOR Sr⁹⁰ ACTIVITY
(2.5 GRAMS OF ASH ARE OBTAINED FROM 70 TO 80 INCISOR CROWNS
AND 14 TO 16 MOLARS). INCISORS AND MOLARS WERE POOLED SEPA-
RATELY BECAUSE OF DIFFERENT CHRONOLOGIC DEVELOPMENTAL PERIODS.
SINCE MILK IS AN IMPORTANT SOURCE OF DIETARY Sr⁹⁰, TEETH OB-
TAINED FROM CHILDREN WHO WERE BOTTLE-FED WERE SEPARATED FROM
THOSE WHO WERE BREAST-FED. ALL TEETH WERE DIVIDED INTO DEN-
TIN AND ENAMEL FRACTIONS IN ORDER TO DETECT DIFFERENCES IN THE
UPTAKE OF Sr⁹⁰ BY THE TWO TISSUES. ANALYTICAL PROCEDURES
ARE DISCUSSED IN DETAIL IN THE 1961 REPORT.

RESULTS

THE LARGEST SAMPLE OF TEETH CAME FROM MONTREAL, AND DATA
FROM THAT AREA ARE TABULATED IN TABLE 1, AND ILLUSTRATED IN

FIGURE 1. THE RESULTS ARE EXPRESSED IN STRONTIUM UNITS ($\mu\mu\text{C Sr}^{90}/\text{GM.CA}$). IMPORTANT FINDINGS ARE BRIEFLY DISCUSSED BELOW.

1. THERE WAS AN INCREASED AMOUNT OF Sr^{90} IN ALL GROUPS FOR EACH YEAR FROM 1949 TO 1955. THIS WAS DUE TO THE UPTAKE OF Sr^{90} IN FOOD AND MILK, WHICH BECAME APPARENT IN 1952 AND INCREASED EACH YEAR UNTIL 1959.
2. SOME Sr^{90} WAS FOUND IN THE DENTIN OF CHILDREN BORN PRIOR TO 1952, WHILE Sr^{90} ACTIVITY IN ENAMEL WAS CLOSE TO BACKGROUND. SINCE 1952 WAS THE FIRST YEAR THAT THE ATMOSPHERE CONTAINED SIGNIFICANT AMOUNTS OF Sr^{90} , THE LACK OF ACTIVITY IN ENAMEL SUGGESTED A MINIMUM OF METABOLIC TURNOVER, AND THE ACTIVITY IN DENTIN WAS DUE TO SECONDARY DENTIN FORMATION.
3. LESS Sr^{90} WAS FOUND IN ENAMEL THAN IN DENTIN FROM THE SAME TEETH. THE DIFFERENCES IN UPTAKE OF Sr^{90} BY ENAMEL AND DENTIN IS SHOWN IN TABLE 2 AND FIGURE 1. WHEN THE PRIMARY TEETH WERE FORMING DURING THE YEARS 1952 TO 1957, THE DIETARY Sr^{90} WAS INCREASING YEARLY. THE DIFFERENCE IN DENTIN AND ENAMEL ACTIVITY REMAINED CONSTANT (FIG. 1 - SHADED PORTION OF BARS), BUT BECAUSE OF THE HIGHER UPTAKE OF Sr^{90} IN THE LATER YEARS, THE PERCENTAGE DIFFERENCE BETWEEN DENTIN AND ENAMEL ACTIVITIES DECREASED YEARLY. IT IS QUITE POSSIBLE THAT THE DIFFERENCE IN ACTIVITY WAS DUE TO DENTIN ADDED TO THE PULPAL SURFACE OF THE TOOTH IN LATER YEARS WHEN THE Sr^{90} ACTIVITY WAS HIGHER, AND AFTER ENAMEL HAD CEASED TO FORM. IF THE DIFFERENCE IN UPTAKE OF THESE TWO TISSUES WAS A FUNCTION OF THE SIZE OF THE APATITE CRYSTAL, AS POSTULATED BY POSNER AND LIKENS¹, THE

DIFFERENCE IN UPTAKE OF Sr^{90} BY DENTIN AND ENAMEL WOULD INCREASE YEARLY.

4. DENTIN AND ENAMEL FROM INCISORS WERE LESS ACTIVE THAN DENTIN AND ENAMEL FROM MOLARS FOR ANY GIVEN YEAR (TABLE 2). THIS IS A REFLECTION OF THE EARLIER CHRONOLOGIC DEVELOPMENT OF INCISORS.

5. CHILDREN WHO WERE BREAST-FED FOR MORE THAN THREE MONTHS HAD LESS Sr^{90} IN THEIR TEETH THAN BOTTLE-FED CHILDREN OF THE SAME AGE (TABLE 1). THIS MAY BE ATTRIBUTED TO DILUTION OF Sr^{90} IN MATERNAL MILK BY Sr RELEASED FROM MATERNAL BONE.

RESULTS FROM THIS EXPERIMENT CANNOT BE COMPARED DIRECTLY WITH OTHER STUDIES (BRYANT ET AL², BUTLER³, AND REISS⁴) BECAUSE IN THESE STUDIES THE TEETH WERE NOT SEPARATED INTO DENTIN AND ENAMEL COMPONENTS. BRYANT ET AL² MEASURED THE UPTAKE OF Sr^{90} IN THIRD PERMANENT MOLARS, WHICH HAVE A VERY LONG DEVELOPMENTAL PERIOD. REISS⁴ USED INCISOR CROWNS EXCLUSIVELY, BUT THEY TEND TO REFLECT THE Sr^{90} ACTIVITY IN MATERNAL BLOOD SERUM. AN INDIRECT COMPARISON WITH REISS⁴ DATA FOR THE YEARS 1952 TO 1954 (TABLE 3) SHOWS THAT Sr^{90} ACTIVITIES FROM INCISOR TEETH FALL BETWEEN THE ACTIVITIES OF ENAMEL AND DENTIN IN THIS EXPERIMENT.

ANALYSIS OF CATTLE TEETH

BROWN ET AL⁵ HAD DOCUMENTED THE POSTNATAL DEVELOPMENT OF CATTLE TEETH AND THE FORMATION OF CROWNS AND PORTIONS OF THE ROOTS CAN BE DATED ACCURATELY. THE DEVELOPMENT OF INCISORS CAN BE DATED WITHIN TWO MONTHS. SEGMENTS OF THE PRIMARY

AND PERMANENT TEETH FORMED AT 6 MONTH INTERVALS ARE REPRESENTED IN FIGS. 2 AND 3. IN ORDER TO TEST THE HYPOTHESIS THAT VARIOUS PORTIONS OF CATTLE TEETH COULD BE USED TO DATE THE UPTAKE OF Sr^{89} AND Sr^{90} BEFORE AND DURING THE RECENT WEAPONS TESTS (1961 AND 1962), THE TEETH WERE EXTRACTED FROM A 21 MONTH OLD HEIFER KILLED ON NOVEMBER 27, 1962. THE ANIMAL'S AGE WAS CONFIRMED BY RADIOGRAPHS. PORTIONS OF THE TEETH REPRESENTING THE PRENATAL, THE 0-6 AND 18-21 MONTH POSTNATAL PERIODS (OR THE DATES JULY TO NOVEMBER, 1960, APRIL TO AUGUST, 1961 AND AUGUST TO NOVEMBER, 1962) WERE MONITORED FOR SR ACTIVITY. THE PRELIMINARY DATA ARE TABULATED IN TABLE 4. POSTNATAL ENAMEL FORMED PRIOR TO SEPTEMBER 1961 WAS SLIGHTLY MORE ACTIVE THAN PRENATAL ENAMEL. POSTNATAL DENTIN FORMED BEFORE SEPTEMBER, 1961, WAS MORE ACTIVE THAN POSTNATAL ENAMEL FROM THE SAME TOOTH. DENTIN FROM ROOTS FORMED IN THE LATE SUMMER AND FALL OF 1962 WAS 3 TIMES MORE ACTIVE THAN DENTIN FORMED IN THE SUMMER OF 1961, THE LATTER DATE REPRESENTING THE BEGINNING OF EXTENSIVE SOVIET AND AMERICAN TESTS. IT IS KNOWN THAT Sr^{90} IN MILK POWDER DROPPED STEADILY FROM 1959 TO 1961, AND ROSE RAPIDLY THROUGHOUT THE SUMMER OF 1962, WHEN THE CATTLE WERE GRAZING ON PASTURE CONTAMINATED WITH FRESH FALLOUT. (BIRD ET AL⁶). THE SR ACTIVITIES IN CATTLE TEETH REFLECTS THIS TREND.

FUTURE RESEARCH

SUFFICIENT DATA HAVE BEEN ACCUMULATED IN THE LAST YEAR TO SUGGEST THAT THE PROJECT WILL YIELD ACCURATE INFORMATION ON THE UPTAKE OF Sr^{90} BY HUMAN DENTAL TISSUES. IT IS

UNFORTUNATE THAT CANADIAN DATA ON Sr^{90} IN RAINFALL, CATTLE BONE AND MILK, AND HUMAN BONE, FOR THE YEARS 1955 TO 1957, ARE NOT SUFFICIENT TO MAKE CORRELATIONS WITH THE TOOTH DATA IN THIS STUDY. THE PRESENT STUDY WILL BECOME MORE MEANINGFUL IF THE CONTENT OF Sr^{90} IN TEETH FORMED IN THE PERIOD 1959 TO 1963 CAN BE COMPARED TO Sr^{90} MEASUREMENTS GATHERED BY THE RADIATION PROTECTION DIVISION OF THE DEPARTMENT OF NATIONAL HEALTH AND WELFARE SINCE 1958. THIS WOULD NECESSITATE AN EXTENSION OF THIS PROJECT FOR AT LEAST 5 YEARS.

MONITORING OF REPOSITORIES OF Sr^{90} IN CATTLE TEETH WHICH CAN BE DATED ACCURATELY WOULD GIVE VALUABLE INFORMATION ABOUT THE UPTAKE OF Sr^{90} IN BOVINE MINERALIZED TISSUES AS COMPARED TO MILK. THIS STEP WOULD SUPPLY AN IMPORTANT LINK IN THE Sr^{90} FOOD CHAIN (GRASS TO CATTLE BONE AND TEETH TO MILK TO MAN).

THE STUDY ON CARBON¹⁴ IN DENTAL TISSUES HAS NOT BEEN CARRIED OUT BECAUSE THE COST OF A C^{14} DATING APPARATUS DID NOT SEEM TO BE JUSTIFIED. THE ROYAL ONTARIO MUSEUM IS PURCHASING A C^{14} DATING UNIT IN 1963, AND IT IS POSSIBLE THAT PERMISSION MAY BE GRANTED TO MEASURE C^{14} OBTAINED FROM THE ORGANIC MATRIX OF DENTIN.

SUMMARY

1. ANALYTICAL METHODS FOR STRONTIUM⁸⁹ & ⁹⁰ AND CALCIUM HAVE BEEN STANDARDIZED.
2. EIGHTY-EIGHT SAMPLES OF POOLED HUMAN TEETH HAVE BEEN ANALYSED.

- (A) THE Sr^{90} CONTENT OF TEETH ($\mu\text{C}/\text{GM. OF CA}$) INCREASED EACH YEAR FROM 1949 TO 1957.
 - (B) LESS Sr^{90} WAS PRESENT IN ENAMEL THAN IN DENTIN FROM THE SAME TEETH.
 - (C) DENTIN AND ENAMEL FROM INCISORS HAD A SMALLER AMOUNT OF Sr^{90} THAN DENTIN AND ENAMEL FROM MOLARS OF CHILDREN BORN IN THE SAME YEAR.
 - (D) TEETH FROM BREAST--FED CHILDREN CONTAINED LESS Sr^{90} THAN TEETH FROM BOTTLE-FED CHILDREN OF THE SAME AGE.
 - (E) SOME Sr^{90} WAS FOUND IN DENTIN, BUT NOT IN ENAMEL OF CHILDREN BORN BEFORE 1951.
3. SAMPLES OF CATTLE TEETH HAVE BEEN ANALYSED.
4. PROGRESS HAS BEEN MADE ON A SUITABLE METHOD FOR THE DETERMINATION OF STABLE STRONTIUM.

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C N H W (RP-3) (RP-4) (RP-5)

YEAR	MOLARS						INCISORS					
	DENTIN			ENAMEL			DENTIN			ENAMEL		
	BOTTLE	0-3 MOS	BREAST 3+ MOS	BOTTLE	0-3 MOS	BREAST 3+ MOS	BOTTLE	0-3mos	BREAST 3+ MOS	BOTTLE	0-3 MOS	BREAST 3+mos
1946- 1949	.067	.067										
1949	.149 .202 .084			.100 .040 .03								
1950	.143 .141 .113		.093(c)	.027 .054		.058(c)						
1951	.192 .188 .156(c)	.142	.133(c)	.058 .061 .070 .054(c)		.035(c)						
1952	.368 .371	.293		.184 .102 .081(c)		.227				.173		
1953	.458 .578			.303 .254		.404 .592 .446				.219 .233		
1954	.958 1.005			.654 .708 .629		.671 .575 .694 .760(c)	.799 .678	.548(c)	.403 .418 .415			
1955	1.398 1.35 1.407			.150 .234		.987 .990 1.137 1.177(c)	1.162(c)	.769(c)	.713 .852			
1957	2.443 2.230											

ALL DATA ARE IN STRONTIUM 90 UNITS ($\mu\mu\text{c SR90/gm.CA}$).
ISOLATION OF SR 90 CARRIED OUT ON 2.5 GM. SAMPLES OF ASH FROM POOLED TEETH

TABLE 2
COMPARISON OF Sr^{90} CONTENT IN DENTIN AND ENAMEL
SAMPLES, 1952 - 1955

YEAR OF BIRTH	MOLARS		INCISORS		DIFFERENCES D-D AND E-E
1952	DENTIN (D)	0.369	DENTIN	0.227	-0.147
	ENAMEL (E)	0.143	ENAMEL	0.173	+0.030
	DIFFERENCE	0.226	DIFFERENCE	0.054	
	% DIFFERENCE	61	% DIFFERENCE	23.8	
1953	DENTIN	0.518	DENTIN	0.481	-0.037
	ENAMEL	0.279	ENAMEL	0.226	-0.053
	DIFFERENCE	0.239	DIFFERENCE	0.225	
	% DIFFERENCE	42	% DIFFERENCE	53	
1954	DENTIN	0.981	DENTIN	0.647	-0.334
	ENAMEL	0.681	ENAMEL	0.412	-0.269
	DIFFERENCE	0.300	DIFFERENCE	0.235	
	% DIFFERENCE	30	% DIFFERENCE	36	
1955	DENTIN	1.385	DENTIN	1.038	-0.347
	ENAMEL	1.192	ENAMEL	0.782	-0.410
	DIFFERENCE	0.193	DIFFERENCE	0.256	
	% DIFFERENCE	14	% DIFFERENCE	24	

ALL ACTIVITIES ARE EXPRESSED AS STRONTIUM⁹⁰ UNITS ($\mu\mu\text{C } \text{Sr}^{90}/\text{GM.CA.}$)

TABLE 3

COMPARISON OF Sr^{90} UPTAKE IN PRIMARY
INCISORS FROM THE STUDIES OF REISS⁴ AND HUNT

	REISS	HUNT		
YEAR	INCISOR CROWNS	INCISOR CROWNS		
		DENTIN	ENAMEL	AVERAGE ENAMEL & DENTIN
	Sr^{90} UNITS	Sr^{90} UNITS	Sr^{90} UNITS	Sr^{90} UNITS
1952	0.196	0.442	0.173	0.297
1953	0.350	0.481	0.226	0.353
1954	0.570	0.647	0.412	0.529

SI - H

TABLE 4

STRONTIUM UNITS IN VARIOUS PORTIONS OF TEETH FROM
21 MONTH OLD HEIFER KILLED NOVEMBER 27, 1962

TOOTH	DATES TISSUE FORMED	Sr ⁹⁰ UNITS
PRIMARY INCISORS PRENATAL ENAMEL	JULY - NOV. 1960	6.37
CROWN 2ND PERM. MOLAR POSTNATAL ENAMEL POSTNATAL DENTIN	APRIL - AUG. 1961	7.17 11.02
		< SOVIET TESTING BEGAN SEPT. 1961
1ST PERM. INCISOR APICAL THIRD OF ROOT		
2ND PERM. INCISOR CERVICAL THIRD OF ROOT	AUG. - NOV. 1962	32.17

TABLE 1
 STRONTIUM UNITS IN VARIOUS PORTIONS OF TEETH FROM
 21 MONTH OLD INFANT AT TWO MONTHS
 FROM NOV. 1952

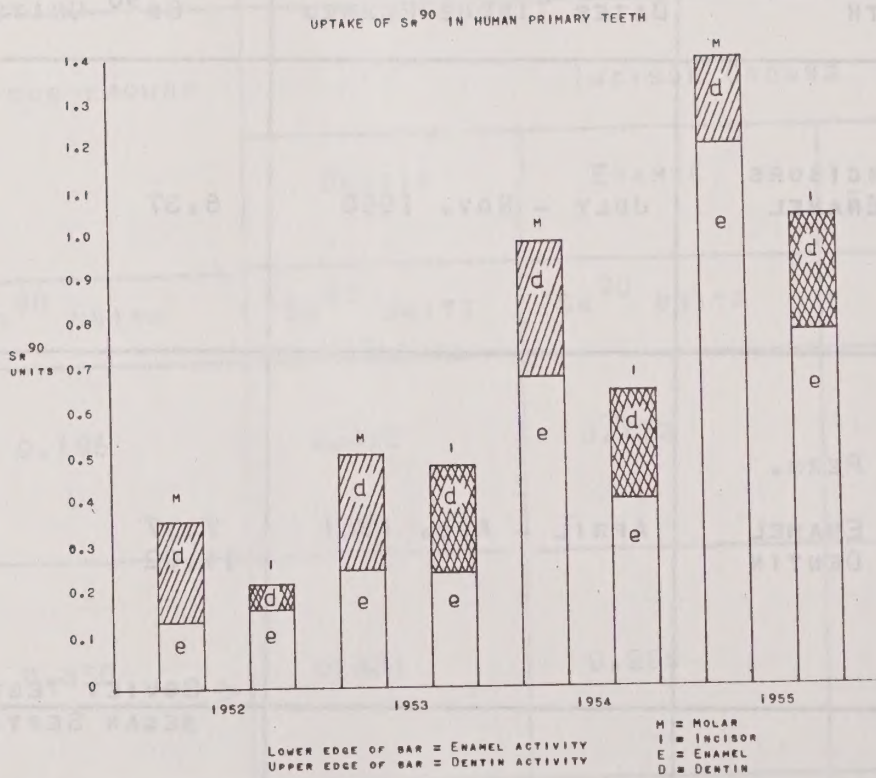


Fig. 1

26 November 1957

REPORT OF A RESEARCH

Dental Survey of Cattle in Great Britain

Name: Omar KHAMIS, M.B., Ph.D., D.F.S., & A. F.R.C.V.S.

Department of Bacteriology and Immunology, Faculty of Medicine and Dentistry.

Institution: Faculty of Dentistry, University of Manitoba.

Title of Project: "A Survey of the Oral Flora of Cattle with special emphasis on the role of the oral flora in the causation of dental disease."

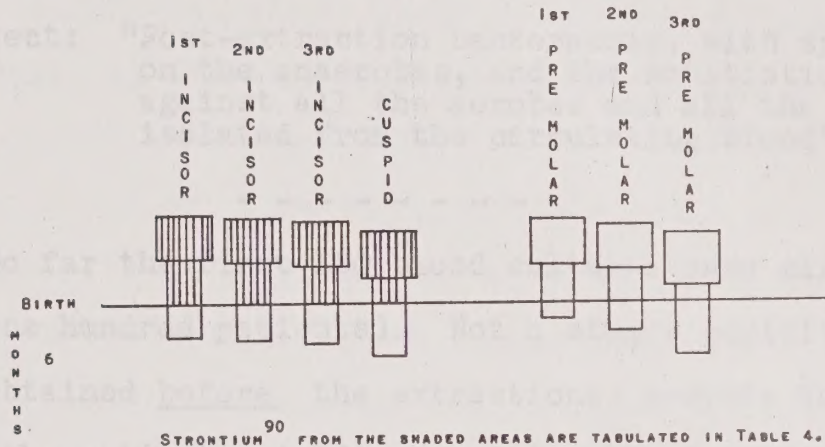


Fig.2

CHRONOLOGY OF PERMANENT TEETH OF CATTLE

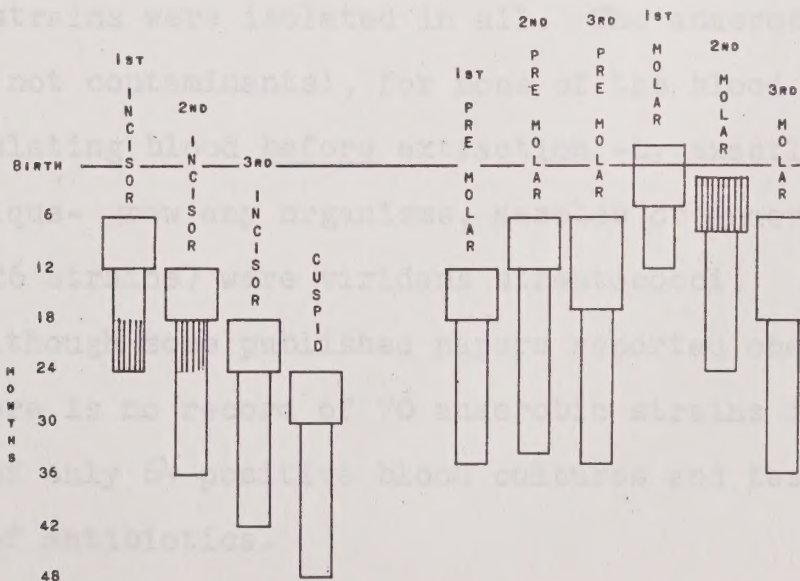


Fig.3

26 November 1962

REPORT OF PROGRESSOne-year supplement to Grant No. DA-101

Name: Omar KHAIRAT, M.D., Ph.D., D.T.M. & H., F.R.P.S.

Department of Bacteriology and Immunology, Faculties of Medicine and Dentistry.

Institution: Faculty of Dentistry, University of Manitoba.

Title of Project: "Post-extraction bacteraemia, with special emphasis on the anaerobes, and the antibiotics active against all the aerobes and all the anaerobes isolated from the circulating blood"

So far the first 100 blood cultures have already been taken (from one hundred patients). Not a single positive blood culture was obtained before the extractions, despite the fact that one third of the patients yielding positive blood cultures (after extraction), had advanced periodontitis and 40% others suffered from a moderate degree of that ailment.

Sixty four of the 100 post-extraction blood cultures were positive. Usually more than one type of organism was isolated from one patient in the majority of the cases. About 50 aerobes and 70 anaerobic strains were isolated in all. The anaerobes (and the aerobes) were not contaminants!, for none of the blood cultures taken from the circulating blood before extraction -by exactly the same optimum technique- grew any organisms, aerobes or anaerobes. Half the aerobes (26 strains) were viridans streptococci.

Although some published papers reported one or more anaerobes, there is no record of 70 anaerobic strains isolated in a small series of only 64 positive blood cultures and tested against a large number of antibiotics.

Antibiotic sensitivity tests of a significant number of the bacteria isolated from these blood cultures (and are thus

potential pathogens as far as endocarditis is concerned) have been performed in search of one single antibiotic that inhibits all the aerobes and all the anaerobes isolated. This is not an easy problem, for the effective antibiotic to be chosen has to be relatively safe, could be given by the mouth as soon as possible before the extraction and must produce high blood levels. The final choice will await the testing of all the strains isolated. This is being done at present.

Once this is settled, the next 100 blood cultures will be taken under cover of the prophylactic antibiotic chosen, and it would be interesting to compare the percentage of the organisms isolated, with that of the first unprotected group. A better plan is to do 200 fresh cases, protecting alternate patients, but this would take two more years.

The full identification and classification study of the bacteria isolated in this work could be the subject of another two-year project and would throw more light on the nature of the bacteria involved in bacterial endocarditis.

APPENDIX J

"THE RELATIONSHIP OF DEFECTS IN TEETH

ASSOCIATED WITH INDUCED STRESSES

TO THE HISTOLOGICAL PATTERN OF TOOTH STRUCTURE."

PROGRESS REPORT

Project: DA - 90

Grantee: Zack Kasloff, Assoc. Prof. Dept. of Restorative Dentistry
(Dental Materials and Fixed Partial Denture Prosthesis)

Institution: Faculty of Dentistry, University of Manitoba.

I The Effects of Various Cutting Instruments on the Hard Structures of the Teeth.

Continuation of the investigation of cracks in teeth induced by various types of cutting instruments has progressed. The report on this phase of the study is embodied in the following paper which was read before the American Dental Association Meeting in Miami, Florida, in October 1962.

Consideration of the effects on tooth structure by rotary cutting instruments has been limited largely to studies of pulpal responses to thermal changes, and surface characteristics of cavity walls and margins.¹⁻⁵ Recently, an investigation was undertaken to determine the relationship of cracks in enamel observed to occur adjacent to cavities, prepared with rotary cutting instruments commonly used in operative procedures.⁶ Results of the study, confined to Class V preparations on lower anterior teeth, indicated that alteration to the hard tissues of teeth could be induced by such instruments.

Crown and bridge procedures employ various rotational cutting devices for reduction and preparation of abutment teeth. Form and magnitude of such preparations differ considerably from that of a simple Class V cavity, therefore, it was decided to determine what effect might occur when common abutment preparations are made with some of these instruments.

PROCEDURE

Preparation Form. A modified partial veneer or three-quarter crown preparation was selected because it includes elements of intracoronal and extracoronal cutting and represents a form which is used clinically for posterior as well as anterior teeth. The selection of this preparation is particularly well suited because the labial or buccal surfaces of the teeth are not reduced to any great extent. These surfaces of the teeth are not reduced to any great extent. These surfaces permit

a relatively large surface of enamel to be examined for changes that might be produced by cutting procedures. The essential features of a partial veneer preparation were embodied in the reduction of the teeth. These included (1) proximal reduction, (2) incisal or occlusal reduction, (3) lingual reduction, (4) proximal groove, (5) incisal or occlusal groove, and (6) a lingual post-hole in anterior teeth. Since only one proximal surface of a tooth was to be examined, no preparation was made on the opposite proximal surface.

To illustrate the type of preparation designed for this study, similar partial veneer preparations were made on enlarged plaster teeth and are reproduced in Figure 1.

Visual Inspection Technique. The technique of specimen preparation, fluorescent dye penetration and inspection under ultraviolet light, adapted for use with natural teeth as reported in a previous study⁶, was utilized in the present investigation. The teeth to be studied are individually embedded in a matrix of artificial stone, allowing the surface under examination to remain exposed. When several surfaces are to be examined, additional matrices are made, one for each surface. This serves as a bed or form which permits a tooth to be removed, treated by whatever means necessary, and returned to its original position.

The success of the dye penetration technique lies in the ability of the dye to penetrate a minute flaw or crevice. An emulsifier assists in removal of residual dye from the surface of the specimen, following which a finely dispersed powder is brought in contact with the tooth. This powder, called a developer, bleeds the dye remaining in a crevice to the surface. When ultraviolet light is applied, a bold fluorescence is revealed wherever a crack exists.

Photography is used to record surface patterns before and after instrumentation. The matrix provides the means of positioning a specimen in the same relation to the camera at all times. It also enables the teeth to be stored in a moist atmosphere

to prevent dehydration.

Specimen Selection and Storage. Ninety freshly extracted human teeth, previously stored in tap water, were obtained from the Oral Surgery Clinic of the University of Manitoba Dental College and associated hospital dental surgery departments. To be consistent with clinical practice, molars, bicuspids, cuspids and laterals were included in the group, and represented teeth commonly utilized as abutments in the posterior and anterior regions of the oral cavity. The majority of specimens were sound. Those that did have carious lesions were only slightly affected. The teeth were cleaned and polished with levigated alumina under moisture, placed in vials containing tap water, and stored in a refrigerator at 40°F.

To determine whether storage in an atmosphere of 40°F might influence the cracking or crazing of enamel surfaces, a control study was carried out with a group of ten teeth. These were subjected to fluorescent dye penetration and inspection prior to storage in the refrigerator and the surfaces were photographed under ultraviolet light. Subsequently, they were soaked in the emulsifier solution and thoroughly washed to remove any residual penetrant dye, and were stored in vials of water at 40°F, for one week. After this interval, the procedure of dye penetration was repeated and the surfaces were again photographed. The results revealed no visible change due to storage at the lower temperature. A representative photograph of these results is shown in Figure 2. Storage at this temperature provided a means of inhibiting bacterial growth, thus eliminating the need of chemicals such as formalin which might affect the properties of the tissues under study.

Power Sources and Cutting Instruments. The power sources employed were an air turbine, and a conventional speed belt driven engine, respectively representing

the ultra-high speed range of 100,000 r.p.m. and above, the high speed range of 60,000 r.p.m. Shapes and sizes of carbide burs and diamond disks, wheels and cylinders, were selected on the basis of their common usage when preparing abutments for partial veneer crowns. These were discarded and replaced frequently to minimize any undesirable effect that might be produced as a result of lost cutting efficiency.

Cutting procedures were performed under a constant spray of water and air, in all speed ranges and with all cutting instruments. Clinical experience with the various cutting devices was the only criterion used in determining the amount of pressure which would be applied.

Photographic Evaluation. Tooth surfaces examined in this investigation included the labial, proximal and lingual of anteriors, and buccal, lingual proximal and occlusal or posteriors. Comparative results of before and after instrumentation, recorded on film in a 1:1 ratio, were mounted on a glass slide and projected on a screen for evaluation of surface changes on the specimens. The terms, no change, slight, moderate and severe, were arbitrarily chosen to describe the amount of cracking that might be produced as a result of abutment preparation. The same criterion for evaluation as used in the previous study of Class V preparations⁶ was applied to this investigation, and is illustrated in Figure 3. Representative films of the surfaces of anterior and posterior teeth, prepared with different cutting devices are shown (Figures 4-9).

RESULTS

Rotational Speeds and Cutting Points. The incidence of crack production in groups of teeth prepared with three power sources and two types of cutting points is summarized on the bar graph in Figure 10. In the ultra-high speed range, diamond and carbide cutting tips each produced changes in 67% of the teeth.

The high speed power source produced changes in 36% of the teeth prepared with diamonds, and 33% of those cut with carbides. Conventional or low speed power sources induced crack formation in 73% of the group cut with diamonds, and 60% in those cut with carbides.

Severity of Cracks. Another bar graph (Figure 11) records the degree of crazing produced in each speed range with both types of cutting tips. In the ultra-high speed range a moderate degree of cracking occurred in the group cut with carbide burs. A similar degree of crazing was seen to occur in the high speed range when carbide cutting points were used. Production of moderate cracking was not evident in the low speed group. All of the groups in the three speed ranges using diamond as well as carbide burs, produced slight degrees of crazing.

Effects on Types of Teeth. The comparative incidence of crack production in anterior teeth as compared to bicuspid and molars is recorded in Figure 12. The degree of severity is also recorded on the same bar graph. All through all types of teeth as groups were subject to crack formation in varying amounts, the molar teeth as a group revealed only slight degrees of cracking. The other two groups produced moderate degrees of crack formation in some of the teeth.

Surfaces Affected. Of all the surfaces examined in the study, labial or buccal surfaces were the least frequently affected and with the mildest degree. Occlusal surfaces also revealed the mildest degree of cracking and were the next lowest as a group in showing any effect. Table 1 provides a breakdown of the surfaces examined, their location, and degree of cracking produced.

DISCUSSION

The teeth used in this study were drawn from a common pool without any attempt to select specimens on the basis of age or nutritional history.

The presence of cracks were interpreted as a surface phenomenon only, their exact clinical significance still unknown. Further work is now in progress to establish their relationship with the histologic structure of the tooth and a method of quantitating the degree of cracking by a method other than personal observation is being developed.

Compared to an earlier study⁶, the Turbojet instrument, representing the high speed range, produced the lowest frequency of cracking. Again, with every speed range, a reduction in severity was evident when diamonds were used in place of carbide burs. Generally, the ultra-high speed group produced changes in as many teeth as did the conventional low speed group. A decrease in severity is also evident when bulkier teeth are prepared. No evidence of severe cracking was observed with any speed group or cutting point when the partial veneer preparation was made.

It would appear that removal of surface enamel tends to reduce the severity of crack production. This would indicate that abutment preparations requiring large areas of enamel reduction such as a full crown preparation might produce fewer crack patterns than a much smaller intracoronal cavity preparation.

SUMMARY

Ninety extracted human teeth in groups of 15 were used to study the effects on teeth when partial veneer crown preparations were made with diamond and carbide cutting instruments operated at different speeds. The technique of fluorescent dye penetration and ultraviolet light inspection was utilized to reveal the initiation or extension of defects on the surfaces of the teeth.

1. The results of this investigation lend further support to the observation that changes of the hard tissues of teeth can be induced by cutting instruments commonly utilized in every-day operative procedures.

2. Partial veneer crown preparations revealed changes with less severity than did small Class V cavity preparations.
3. Because partial veneer preparations involved more surface enamel removal than do small intracoronal preparations, a reduced effect on the residual surfaces could be expected.
4. Diamond cutting instruments were less severe in their effect than carbide burs.
5. The high speed water turbine powered instrument produced fewer cracks in teeth than the air turbine or low speed powered instruments.
6. No direct correlation was evident between various speeds of rotation and frequency of crack occurrence.

11 Adaptation of the Tooth Fluorometer.

An earlier study conducted by the author⁶ suggested the possibility of developing a quantitative method of measuring the cracking effect on tooth structure induced by various rotary cutting devices. Under provisions of a grant given the previous year, consultation was arranged with A.F. Forziati at the National Bureau of Standards, Washington D.C., where the grantee was able to study the Tooth Fluorometer developed by Forziati and Associates⁷. This has resulted in the adaptation of elements of the Tooth Fluorometer to the present study so that both a photographic evaluation and a quantitative measurement could be obtained at the same time.

Apparatus: The apparatus consists of an ultra-violet light source (3650°A region) with a filter to block off visible light, a lens to collect the emitted fluorescence of the tooth specimen under the UV light, a photoelectric cell connected to a photomultiplier which measures the intensity of the fluorescence, and a camera to record the surface pattern of the tooth. All tooth specimens to be studied are subjected to the fluorescent dye penetration technique before and after cutting procedures so that comparative evaluations can be made from recorded results obtained before and after instrumentation. A fan is used as a cooling device for the ultra-violet lamp because the heat which builds up in the metal cone is of sufficient magnitude to cause rapid deterioration of the lamp. Figure 13 illustrates the components of the assembly.

Optical System: The optical system is illustrated in figure 14. A mercury reflector spot lamp provides the UV light source. The attached filter permits light in the 3650°A region only to pass through. A cone fitted to the lamp, concentrates the light on the object fitted at the base of the microscope. Fluorescent light emitted from the tooth is then collected by the 135 m.m. focal

length lens. A Wratten 2A filter screens out all reflected UV light allowing only fluorescent light to pass through. The light is then passed through a Zeiss basic body II equipped with a 35 m.m. camera, a focussing eyepiece and a photoelectric cell. A mirror, capable of being rotated through a 90° angle by means of a lever, directs the light to either of the three elements, enabling the operator to properly focus the object, read the intensity of the emitted fluorescence and record the surface under study on film.

Calibration: To stabilize the units, it was found necessary to turn on the UV lamp, fan and photomultiplier for 90 minutes before the start of an experiment. A 2"x2" square of Corning glass CS-3-79, gives a reading of 40 on the X 100 scale of the photomultiplier. This was used as a standard to maintain a constant quality of UV light. Frequent checking indicated that the unit was stable within the variation of $\pm \frac{1}{2}$ a division.

Method of Fluorescence Measurement: The tooth specimens were subjected to the usual dye penetration procedure before and after cutting procedures and readings on the photomultiplier were recorded at the same time that photographs were obtained. The values of each surface of the tooth were added together and the resultant figure was used as the measurement of fluorescence. The values of before and after cutting procedures were expressed as a per cent increase or decrease in fluorescence value. Several preliminary trial groups of teeth were used to establish the procedure to be employed. Finally, 5 out of the 6 groups evaluated and studied photographically in Part I were measured for fluorescence with the photomultiplier.

Results: Many problems seem to be present at this time which interfere with the ability to relate what is seen photographically with the values obtained by means of the tooth fluorometer. With time, there appears to be a greater amount

of fluorescent dye absorption in the tooth substance proper, yielding higher readings than obtained initially. Another factor which has to be accounted for is the reduced mass of the tooth crown as a result of cutting procedures, so that the fluorescent readings are not comparable. It will be necessary to develop a technique which will stabilize the amount of fluorescent dye absorption in the tooth and to account for the reduced mass of the tooth due to cutting operations, before reasonable comparisons can be made. This portion of the study is still in a developmental stage.

LegendsFigure 1.

Plaster reproductions showing partial veneer crown preparations on central incisor, bicuspid and molar.

Figure 2.

Representative photographs of the control study showing no change in crack patterns resulting from storage at 40°F. A, labial view; B, proximal view.

Figure 3.

Criterion for evaluation. A, no change; B, slight degree; C, moderate degree; D, severe degree.

Figure 4.

Photograph illustrating crack patterns before and after cutting on an anterior tooth with a diamond point and an air turbine instrument.

Figure 5.

A similar illustration when carbide bur was used.

Figure 6.

A bicuspid, prepared with a diamond point and water turbine instrument.

Figure 7.

A bicuspid, prepared with a carbide bur and conventional handpiece.

Figure 8.

A molar, prepared with a diamond point and a water turbine instrument.

Figure 9.

A molar, prepared with a carbide bur and water turbine instrument.

Figure 10.

A bar graph showing percent incidence of crack production with three speeds of rotary cutting instruments using diamond points and carbide burs.

Figure 11.

A bar graph relating the severity of crack production and incidence of occurrence in the three speed ranges and two types of cutting points.

Figure 12.

A bar graph showing percent incidence and degree of cracking occurring with anterior, bicuspid and molar teeth.

Figure 13.

Photomacrographic assembly.

Figure 14.

Optical System.

Table 1.

Surfaces affected and degree of cracking produced.

FIGURE 1

References:

1. Peyton, F.A. and Henry, E.E. The Effect of High Speed Burs, Diamond Instruments and Airbrasive in Cutting Tooth Tissue, J.A.D.A. 49:426, 1954.
2. Charbeneau, G.T. and Peyton, F.A. Observations from Shadowed Collodion Replicas of Teeth with Amalgam Restorations, J.D. Res. 36:623, 1957.
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4. Peyton, F.A. Response to Shaping Cavities with Modern High Speed Instruments, New York J.Dent. 18:262, 1958.
5. Charbeneau, G.T. and Peyton, F.A. Some Effects of Cavity Instrumentation on the Adaptation of Gold Castings and Amalgam. J.Pros.Dent. 8:514, 1958.
6. Kasloff, Z. A Method of Demonstrating, In vitro, the Effects of Various Cutting Instruments on Tooth Structure. Thesis, Indiana University, 1960.
7. Kimpula, J.W.; Barone, J.J. and Forziati, A.F. Tooth Fluorometer. J.D.Res. 30: 753, 1960 (abstract).

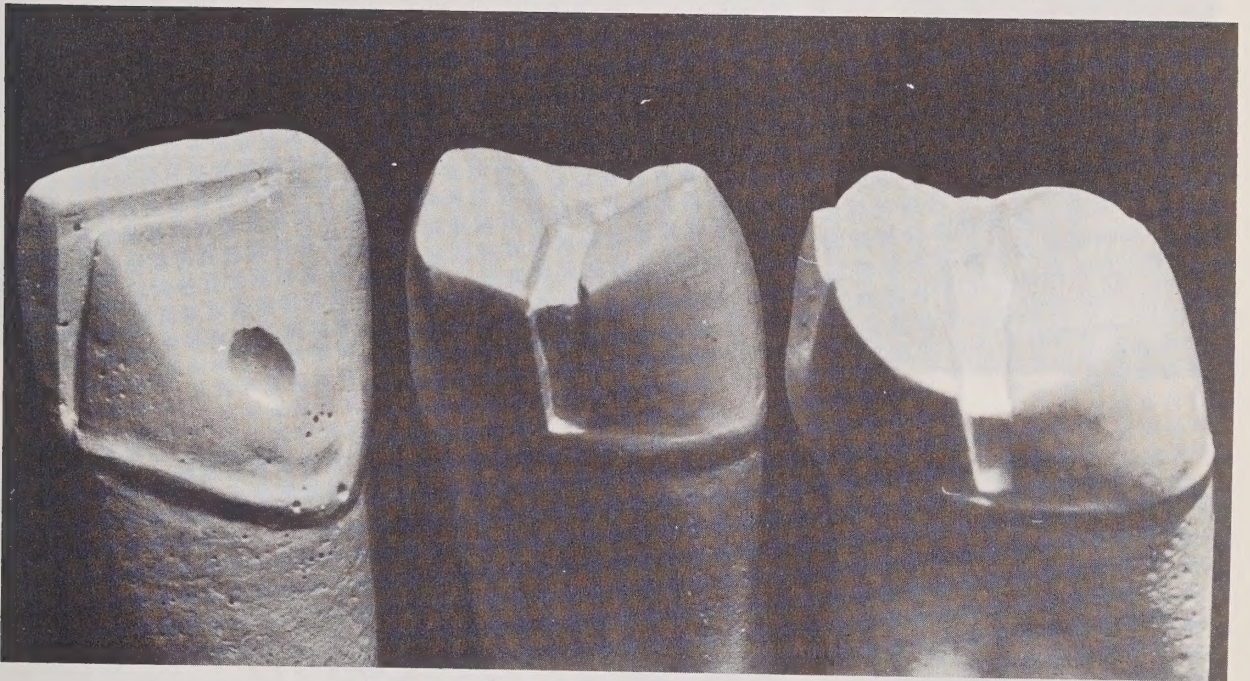


FIGURE I.

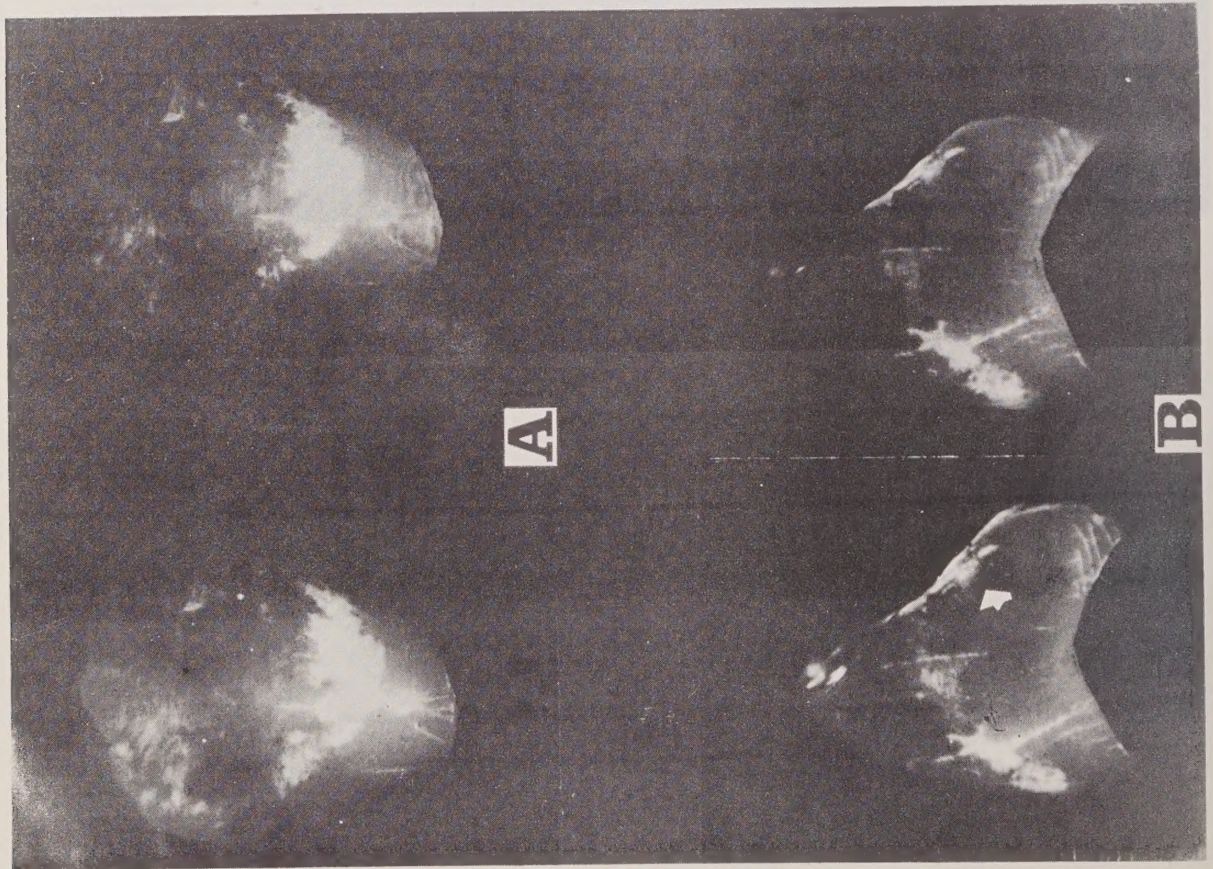


FIGURE 2.

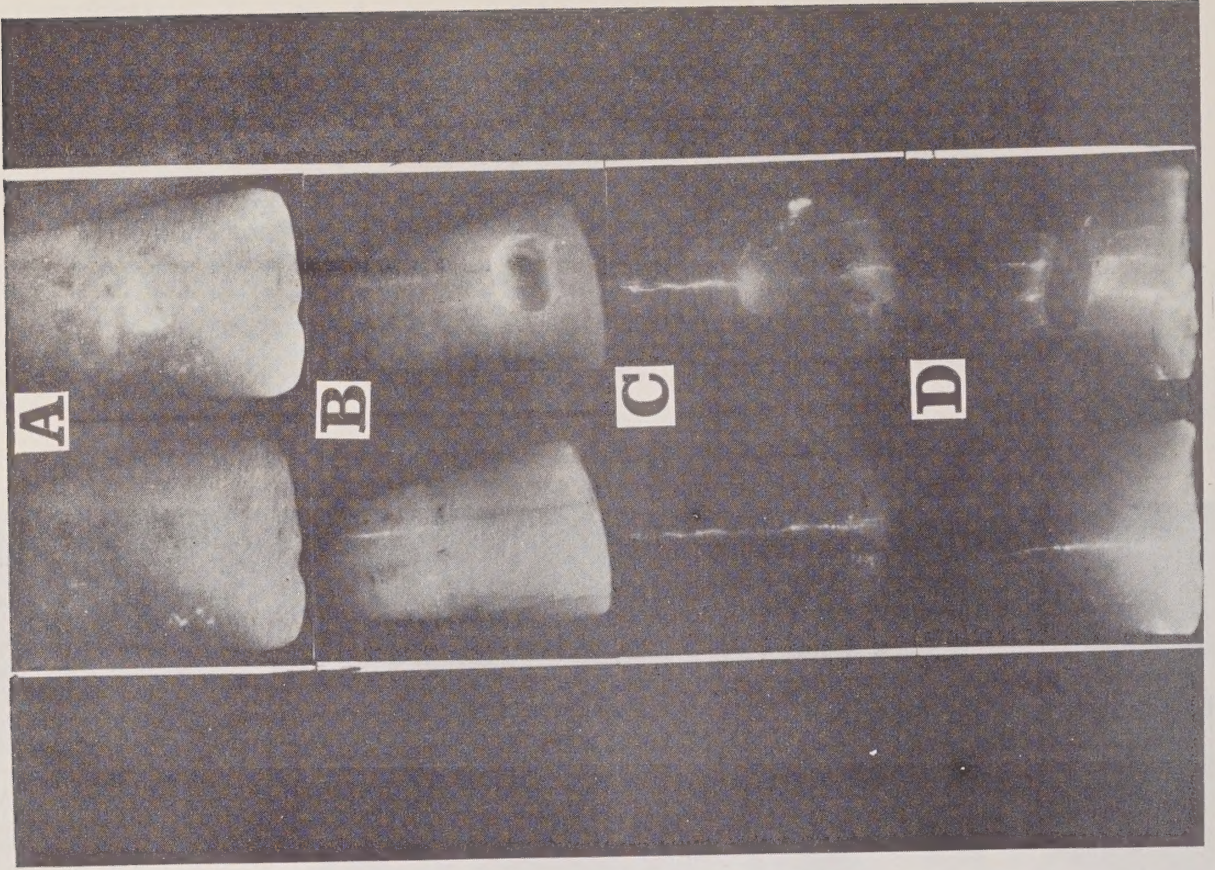


FIGURE 3.

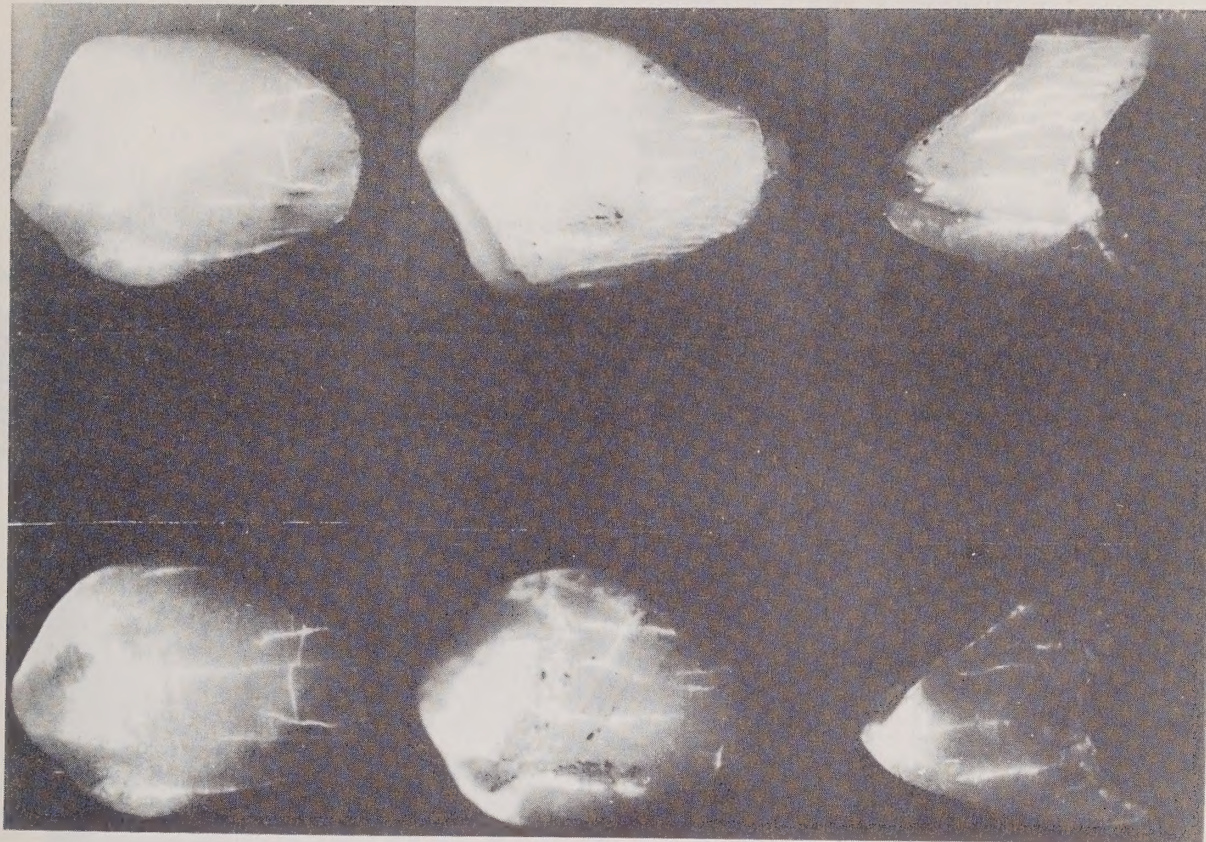


FIGURE 5.

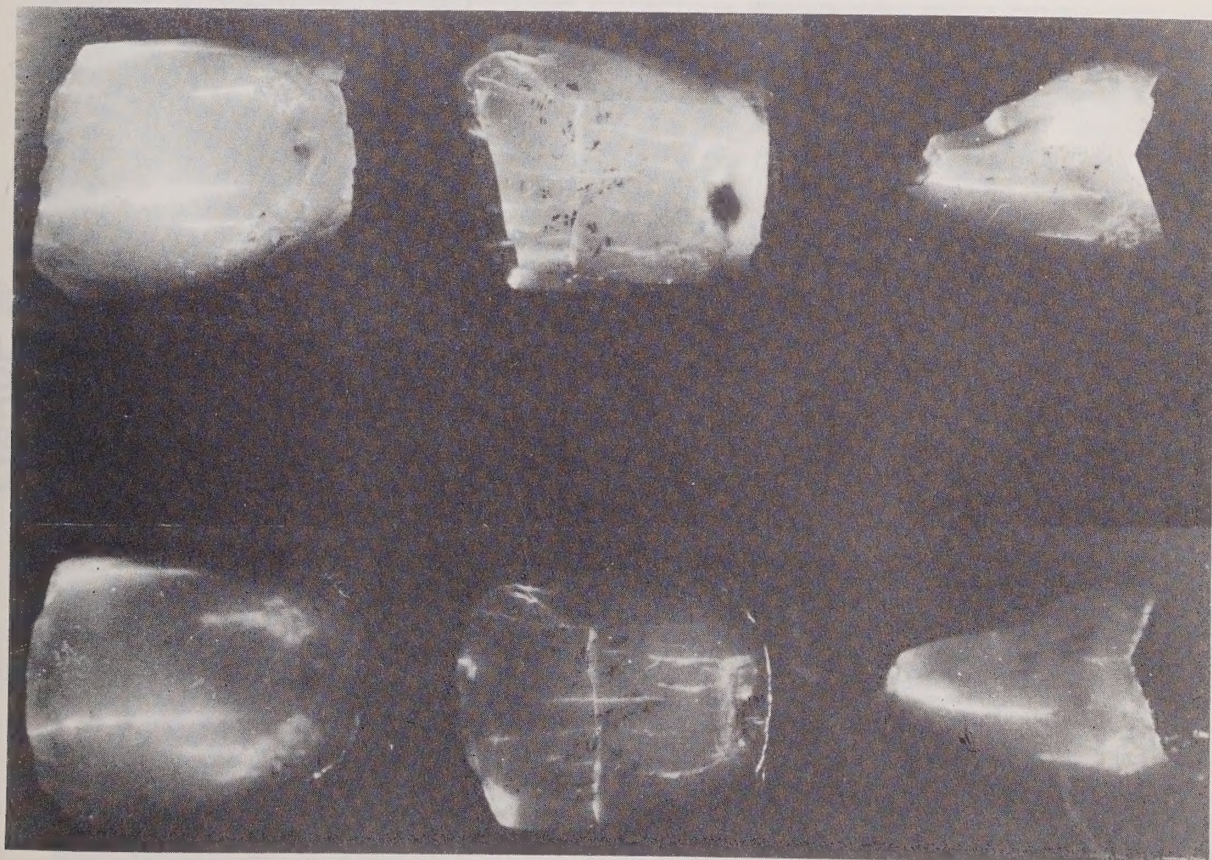


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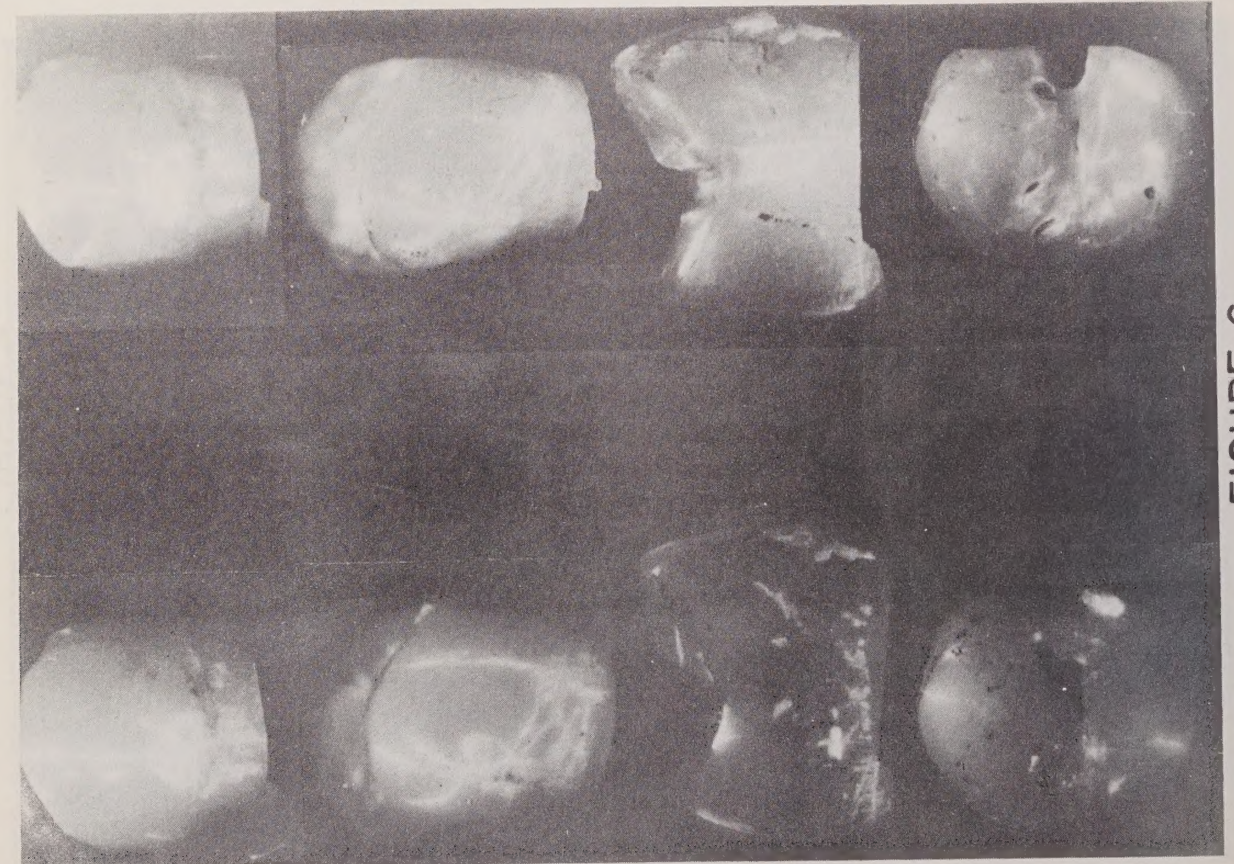


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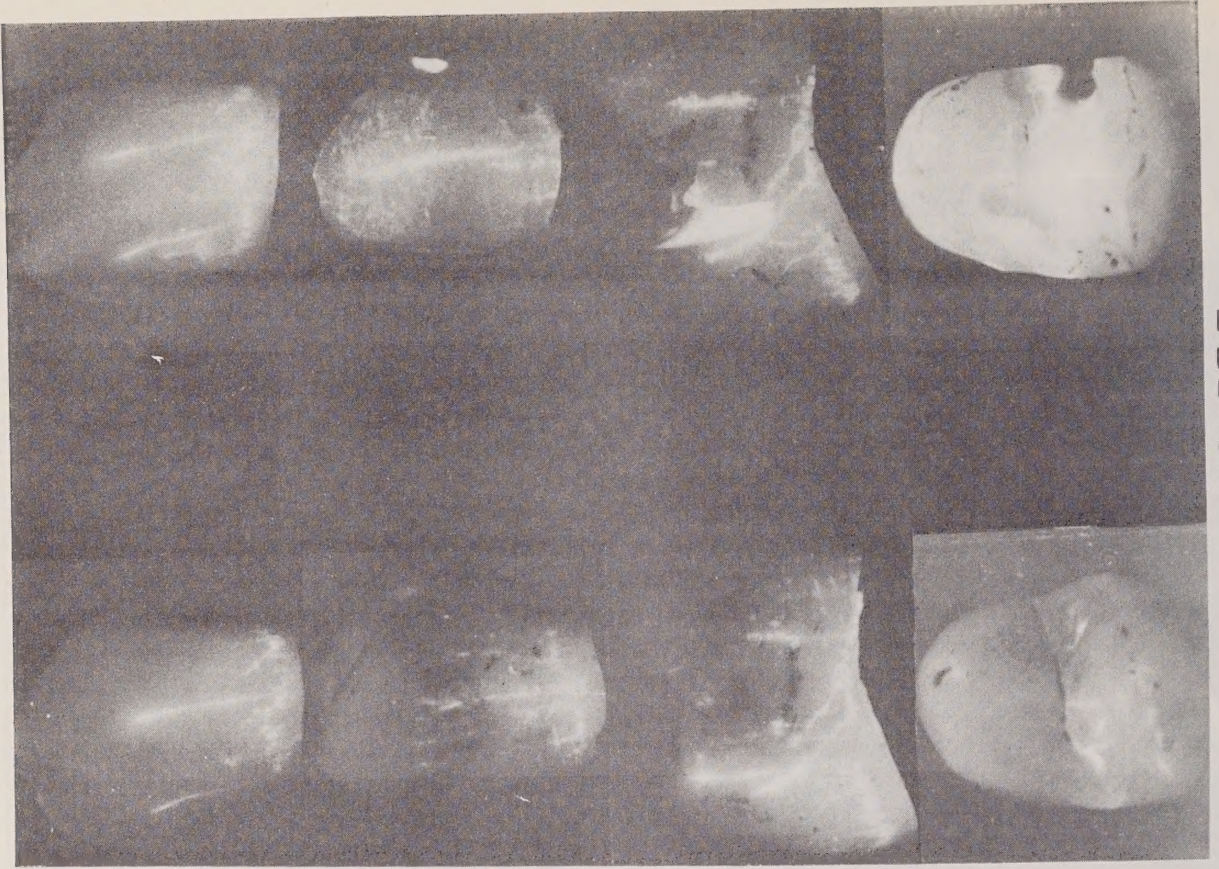


FIGURE 7.

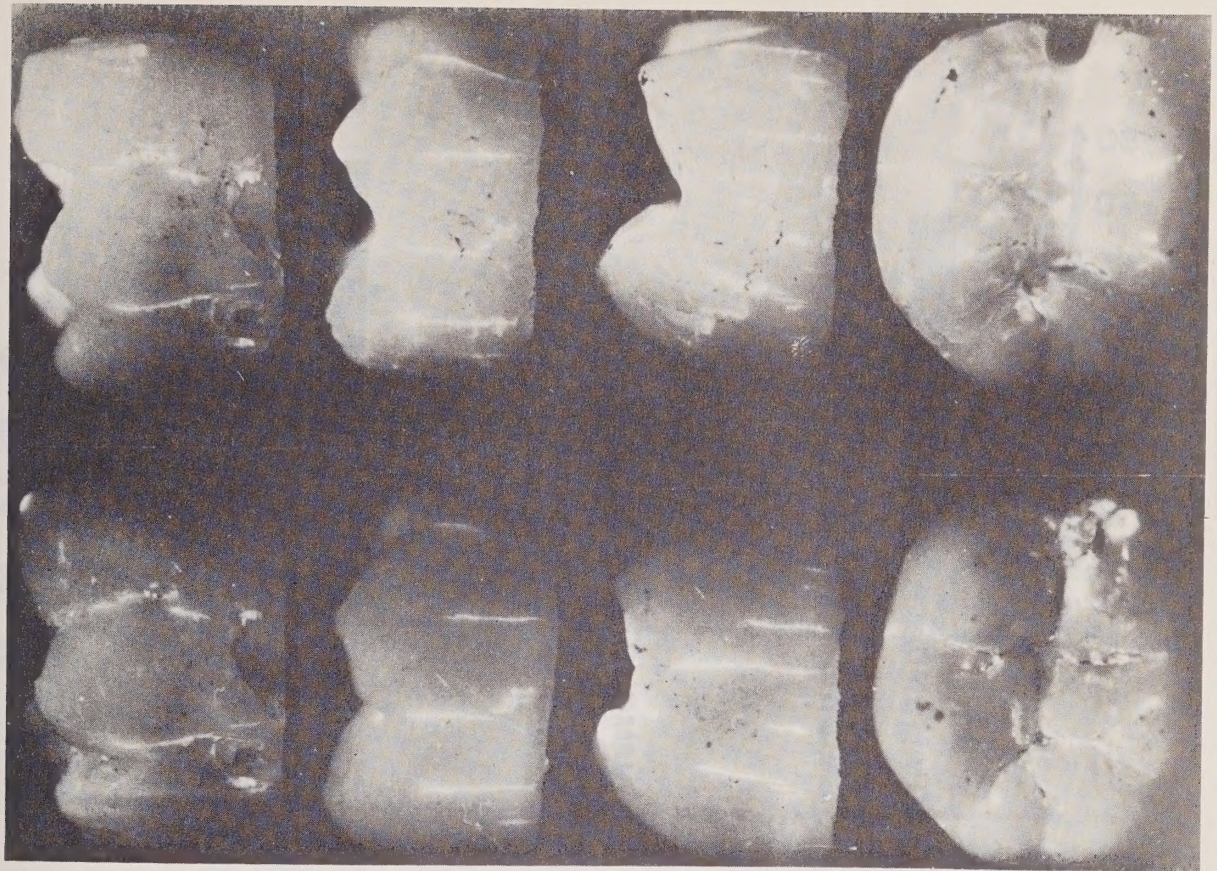


FIGURE 9.

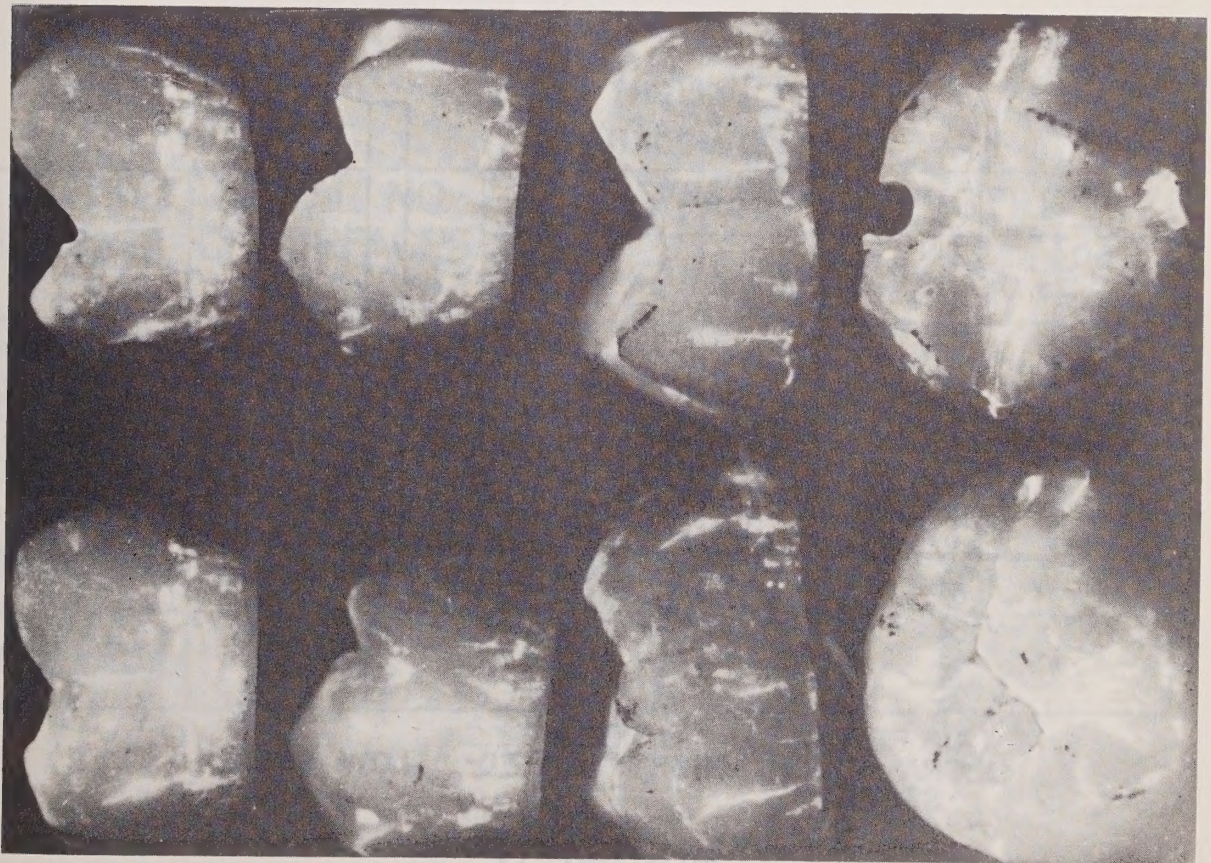


FIGURE 8.

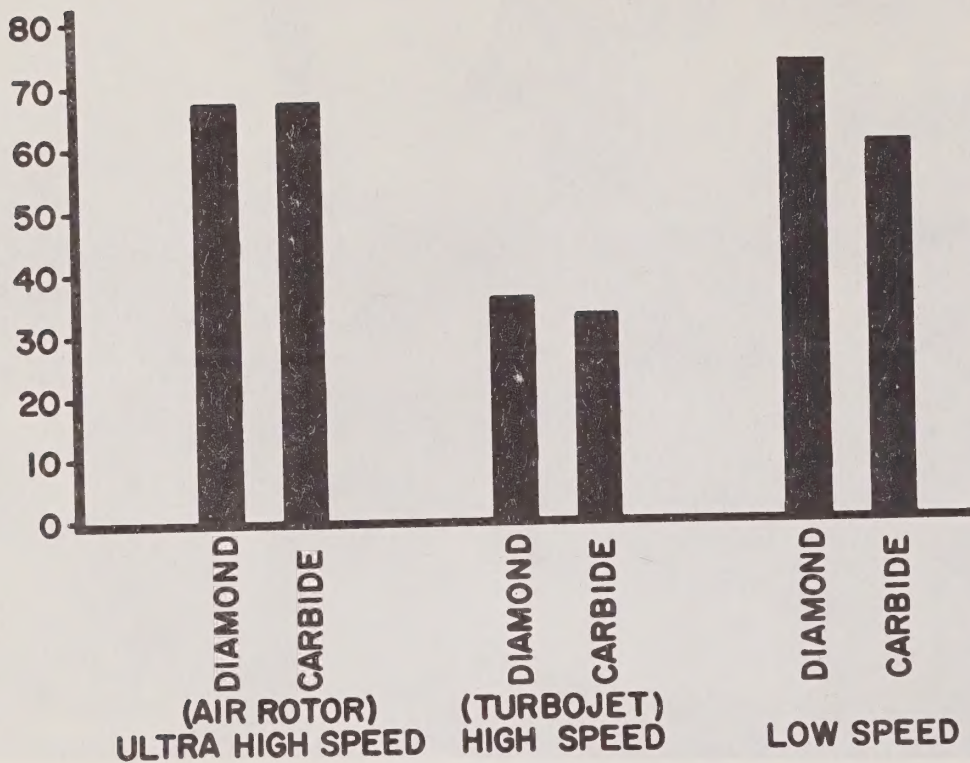


FIGURE 10.

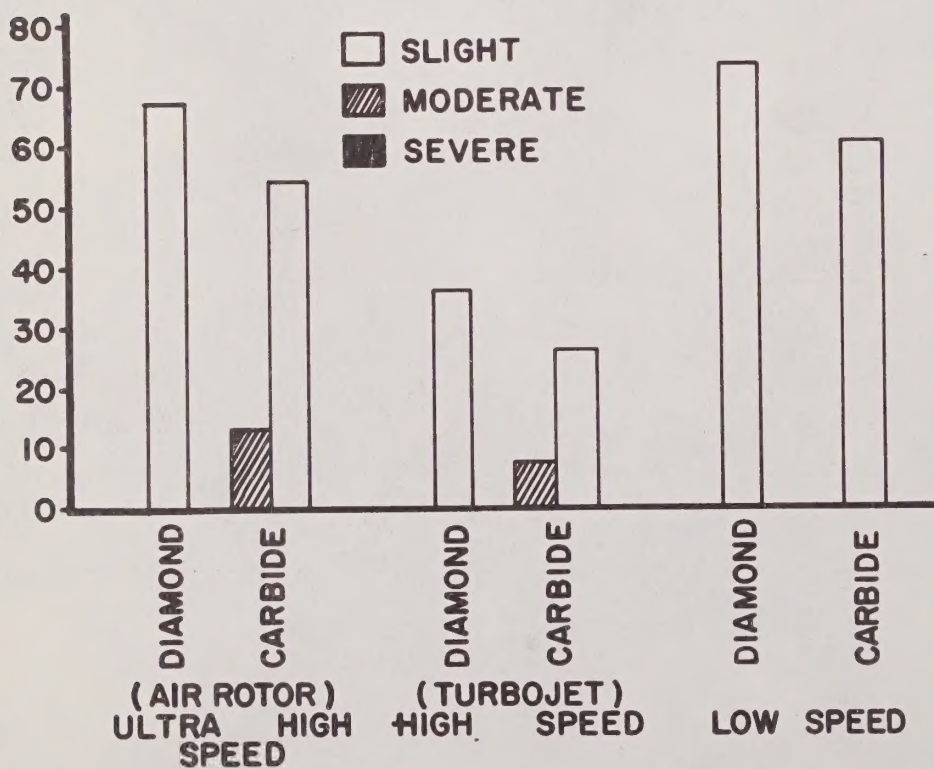


FIGURE 11.

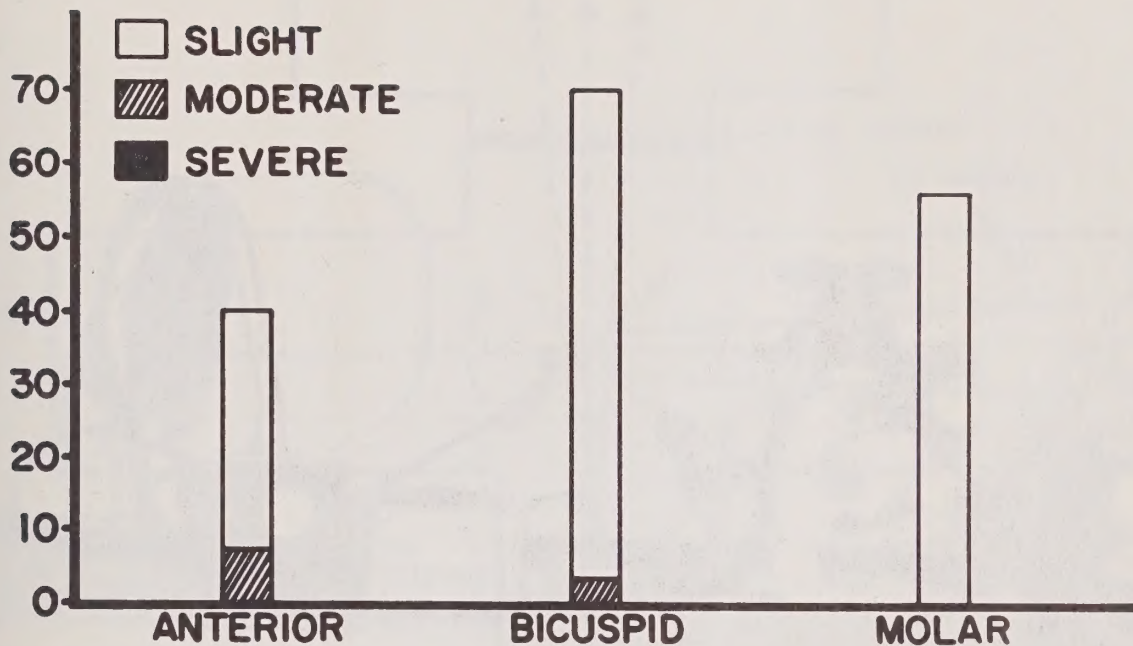


FIGURE 12.

SURFACE	TOTAL NO. OF SURFACES EXAMINED	SURFACES AFFECTED		
		SLIGHT	MODERATE	SEVERE
LABIAL or BUCCAL	89	5		
LINGUAL	89	34	1	
PROXIMAL	89	20	2	
OCCLUSAL	48	11		

TABLE I.

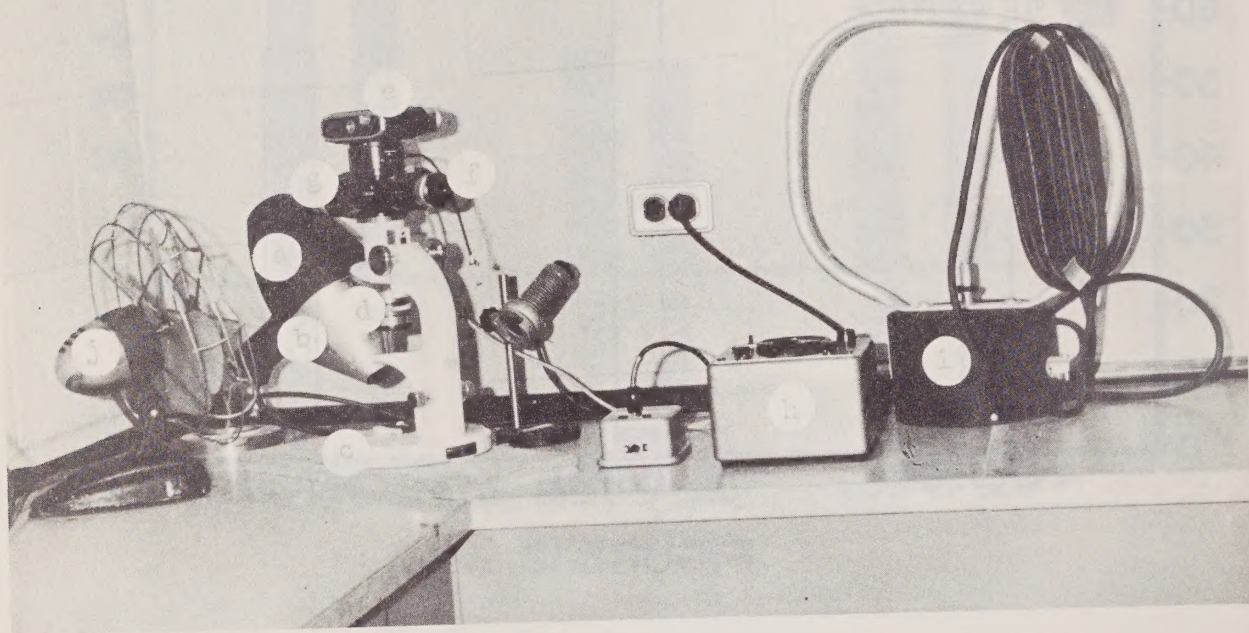


FIGURE 13.

Fig. 13. Tooth Fluorometer and Photomacrography Assembly.

- (a) Ultra-violet lamp with filter
- (b) Metal cone
- (c) Stone matrix with plexiglass mounting plate
- (d) 135 mm. focal length objective
- (e) 35 mm. camera
- (f) Focusing eyepiece
- (g) Zeiss basic body with photoelectric cell at rear
- (h) Photomultiplier
- (i) Transformer for UV lamp
- (j) Fan

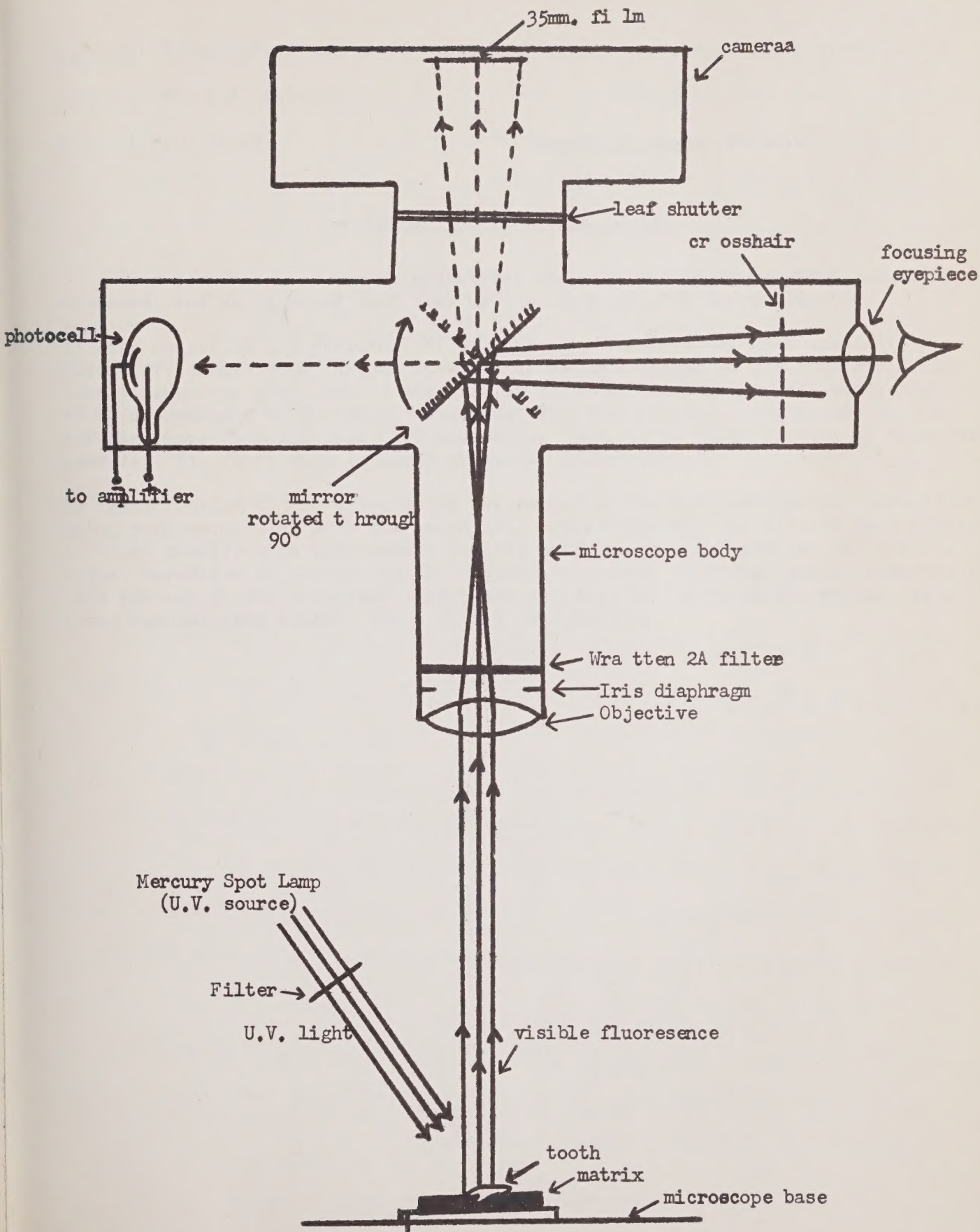


Fig. 14

Optical system

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

January 1963

Subject: Formation of matrix of enamel and dentin (continuation of grant DA 23).Grantee: Dr. C.P. LeblondProject No.: DA 23Amount of grant: \$6500.00SUMMARY OF WORK DONE IN 1962

This year has been one of consolidation, confirmation of previous results was obtained; and it is hoped that they will be soon written for publication.

- 1) With regard to the structure of ameloblasts, the terminal bars and intracytoplasmic filaments were further examined at various stages of the life cycle. The long filamentous tube seen in the axis of secretory ameloblasts has been shown to be a component of the Golgi apparatus. Electron microscope work done on the terminal bars revealed that they are mainly composed of thick bundles of filaments (see fig. 8). (Work with Y. Clermont and E. Kallenbach).
- 2) The formation of the proteins of the enamel matrix has been further investigated using radioautography with the amino acid tryptophane- H^3 . The results were similar to those described in last year's report, with leucine- and proline- H^3 , that is, first, formation of protein in the supranuclear zone; secondly, rapid secretion of this protein to the preenamel layer; and thirdly, diffusion of the protein into young and maturing enamel. (Work with H. Warshawsky).

REPORT ON

WORK done in 1962 by E. Kallenbach (with the help of R.E. Gibbons)

In addition to continuing work with the tannic acid, phosphomolybdic acid and amido black (TPA) technique, we used osmic acid and plastic embedding to investigate the enamel organ of adult rat incisors for study with the light and the electron microscope. Lower incisors were fixed in Palade's fixative by immersion. The head region of one animal was fixed with Palade's fluid by Palay's perfusion technique (Palay et al, 1962). Pieces from each region of the enamel organ were embedded in Epon according to Luft (1961). For light microscopy, sections were cut at $1/2 \mu$ on a Porter-Blum microtome with glass knives, stained with toluidine blue and mounted as ordinary histological slides (under instruction of Dr. E. Sandborn). For electron microscopy, sections were cut at $500 \text{ \AA}$ (collaboration of Dr. E. Sandborn). The quality of the perfused tissues was found to be superior to that of the tissues fixed by immersion. The terminal bars, desmosomes and filamentous structures are described in figure 1 for the immature ameloblast, in figure 2 for secretory ameloblasts, that is, at the stage of enamel matrix formation, in figures 3 and 4 for the post secretory ameloblasts. Desmosomes and other intercellular attachments were found to exist between ameloblasts and the underlying intermediate layer in post secretory (figs. 3, 4 and 6), but not in secretory ameloblasts (figs. 2 and 5). With the electron microscope the apical and basal terminal bars of secretory ameloblasts turned out to be chiefly composed of intracellular bundles of tonofibrils (fig. 8). The Golgi apparatus was found to have an elongated tubular shape and extended between nucleus up to the apex of the cell.

During enamel maturation, the ameloblasts developed a complex microvillous border towards the enamel (fig. 7). Bundles of tonofibrils were running longitudinally in the ameloblasts (figs. 3 and 4).

(under C.P. Leblond;
Department of Anatomy,
McGill University)

LEGEND OF FIGURES

Figs. 1-4 Schematic drawing of longitudinal fibrils and desmosomes of ameloblasts. The apex (on the side of enamel matrix) is at top. The base (in contact with the intermediate layer) is below.

Fig. 1 Immature ameloblast.

Note desmosomes at top and basal terminal bars^(TB). Longitudinal fibrils may also be seen.

Fig. 2 Secretory ameloblasts.

Heavy apical terminal bars and standard-size/basal terminal bars. (The supra nuclear tube is mainly composed of Golgi material; and mitochondria are seen in the base of the cell).

Fig. 3 Early post-secretory ameloblasts.

Note position of apical and basal terminal bars. In addition, both the apical and basal surfaces have desmosomes^(D).

Fig. 4 Late post secretory ameloblasts.

There are apical and basal terminal bars, as well as apical and basal desmosomes. Finally lateral desmosomes are seen in the apical region.

Fig. 5 Base of secretory ameloblasts and adjacent cells, cut obliquely, OsO_4 fixed, $1/2 \mu$ section, stained with toluidine blue. x 1000

N, nucleus of ameloblast. TB, basal terminal bar of ameloblast. Unlabelled arrows: areas of contact between basal end of ameloblasts and cells of intermediate layer. Note absence of desmosomes or other intercellular attachment sites. Contrast with the surface of contact between cells of intermediate layer, where numerous desmosomes are seen (D).

Fig. 6 Base of post secretory ameloblasts and adjacent cells, cut obliquely. Same technique as above. x 1000

N, nucleus of ameloblast. TB, basal terminal bar of ameloblast. Unlabelled arrows: areas of contact between basal ends of ameloblasts and intermediate layer cells; note numerous desmosomes.

Fig. 7 Apex of post secretory ameloblasts. OsO_4 fixation. $\times 20,000$
MV, microvillous border facing maturing enamel. Apical terminal bars
at TB.

Fig. 8 Base of secretory ameloblasts and adjacent cells. OsO_4 fixation.
 $\times 20,000$
N, nucleus of ameloblast. TB, basal terminal bar of ameloblasts. Un-
labelled arrows: areas of contact between ameloblasts and intermediate
layer cells. Note absence of desmosomes.

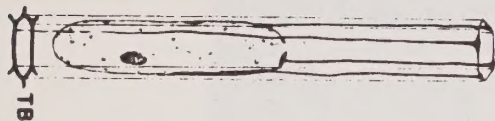
Immature

Secretory

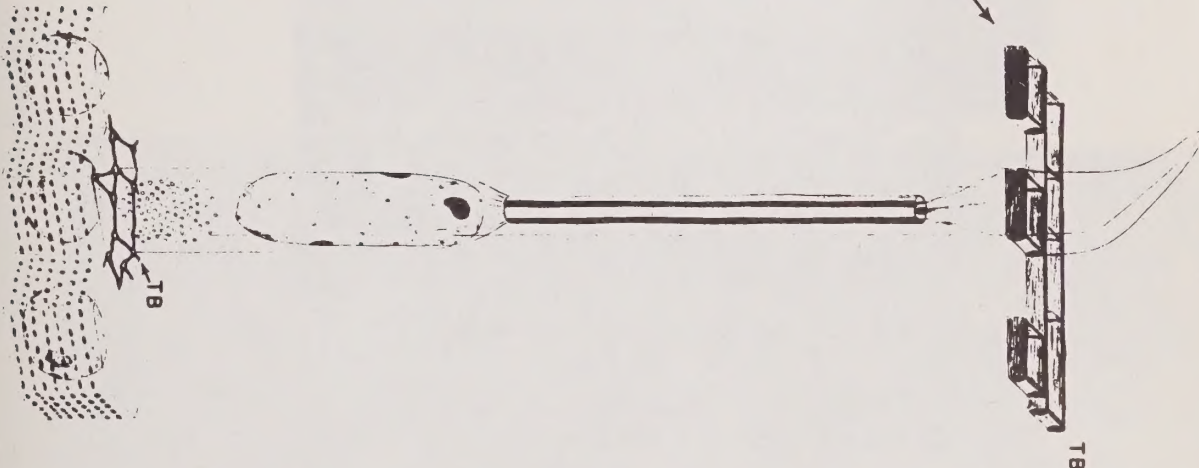
Post - secretory

Apical
region

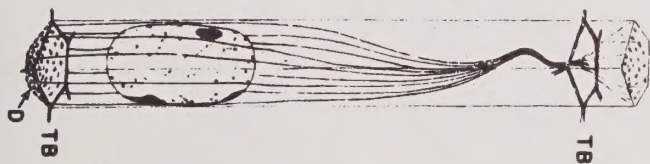
Base



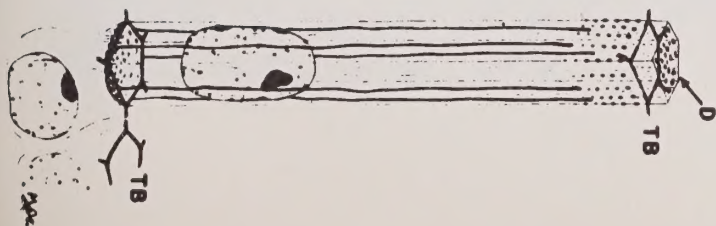
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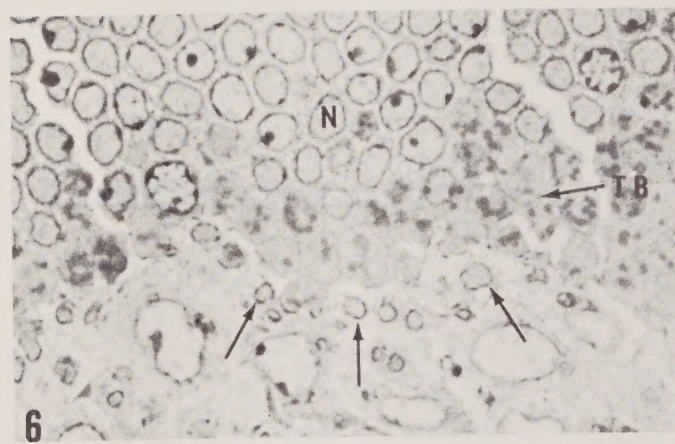
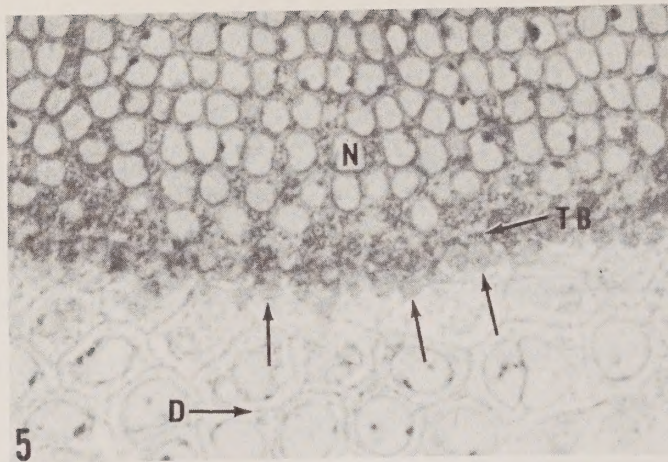
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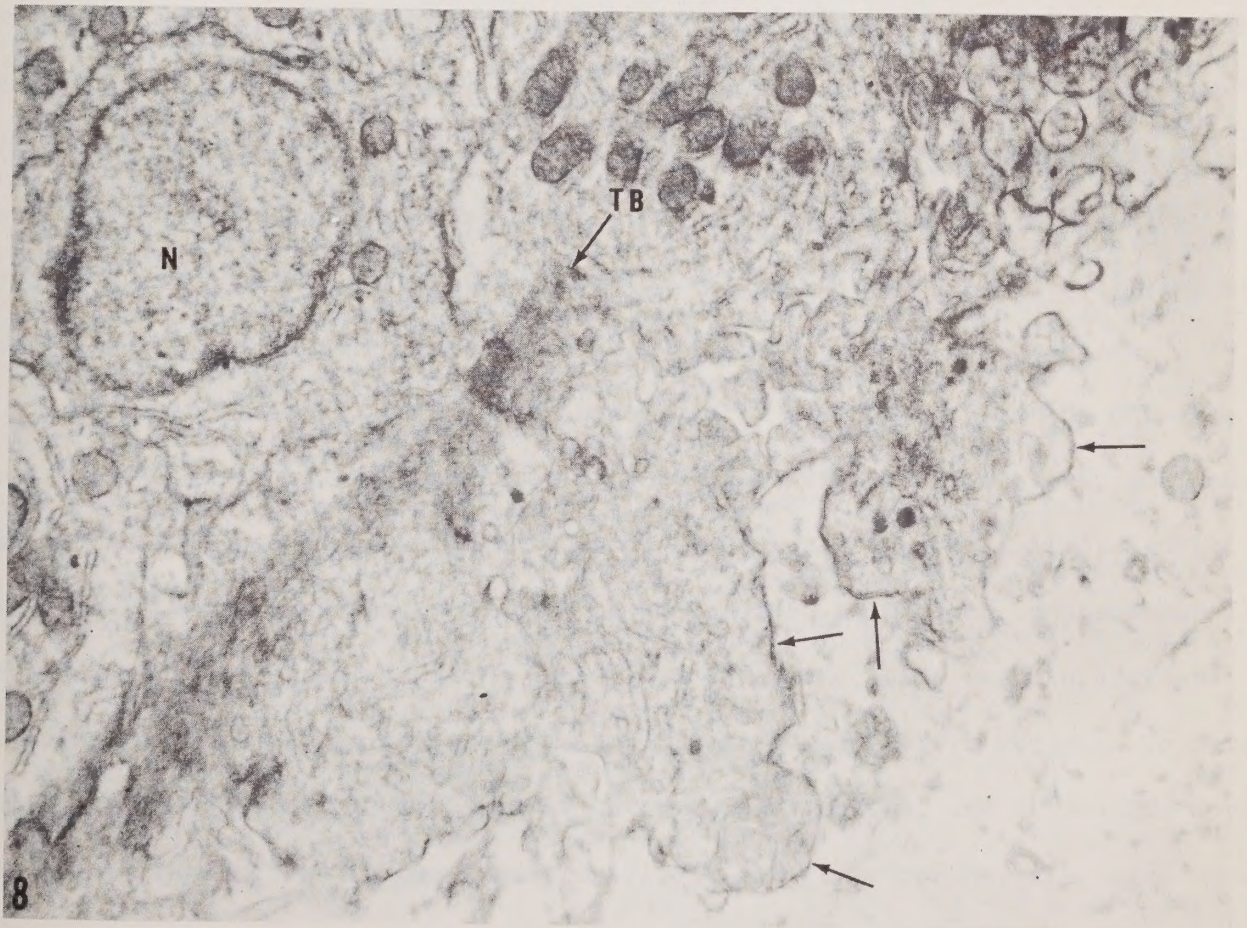


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RADIOAUTOGRAPHIC VISUALIZATION OF ENAMELMATRIX PROTEIN FORMATION USING TRYPTOPHANE-H³

H. Warshawsky, L. Puchinger and C.P. Leblond

Department of Anatomy, McGill University

Montreal, Canada

Project D A 23

The radioautographic technique has been applied to the study of enamel matrix protein formation using various labelled amino acids as precursors of these proteins. Thus, using methionine labelled with S³⁵ or H³, Karpishka et al (1959) found that soon after injection (30 minutes) two bands of radioautographic reactions were present over the enamel organ of mice, viz. one located over the supranuclear region of the ameloblasts, that is, a band extending from the cell nucleus to a region just below the apical terminal bars, and a second band located over the Tomes processes and the preenamel. These results have been repeatedly confirmed using various labelled amino acids, such as leucine-H³ and proline-H³ (see last year's report). However, at earlier times after injection (10 minutes) only the supranuclear band was present (Leblond and Smallwood, 1961). This provided evidence to suggest that proteins were elaborated in the ameloblasts, and were rapidly secreted across the cell membrane to form the preenamel.

The amino acid composition of enamel matrix has been studied by Glimcker et al (1961) in fetal bovine enamel matrix. Their results showed that methionine accounted for 48.6 amino acid residues per 1000 total residues. Also, leucine made up 97.2 residues, and proline, the most common amino acid, contributed 215.0 residues per 1000 total residues. Thus, all the amino acids used in previous studies were present in rather large amounts in enamel matrix protein. To obtain a further control on the accuracy of the previous results, and to examine more closely the reactions in the enamel matrix itself, tryptophane, the least common

amino acid present (1.78 residues per 1000 total residues), labelled with tritium (H^3), was selected for investigation.

MATERIALS AND METHODS

Male Sprague-Dawley rats, weighing about 100 gms, were given a single intraperitoneal injection of 2.5 μ c per gram of body weight of DL-tryptophane- H^3 (Radiochemical Centre, Amersham, England; specific activity: 658 mc/mM). The animals were sacrificed in pairs, very close together under ether anesthesia, at 5 and 15 minutes, 1 and 12 hours, and 4 days after injection. The upper and lower incisors were dissected from the jaws immediately after anesthesia induction and fixed for 48 hours in 2 changes of Bouin fixative, placed in 70% alcohol and decalcified in Versene. Five micra hematoxylin-eosin stained sagittal sections were radioautographed (Kopriwa and Leblond, 1961) and exposed for 97 days.

Quantitative methods

Well sectioned teeth were chosen for quantitative estimations. Counts were made over the ameloblasts and the adjacent matrix of the enamel organ, beginning at the root, or formative end, and extending incisally up to the point where enamel matrix was completely lost. Such counts included ameloblasts at the various stages of their life cycle, that is, immature, secretory and post secretory ameloblasts.

Those areas counted showed a relatively long and uninterrupted row of longitudinally sectioned ameloblasts, as well as a fairly intact enamel matrix. Distorted areas were omitted from the counts. Grain counts were made under an oil immersion objective using a Whipple micrometer ocular grid to delimit a known area of the section. The grid measured 100 x 100 μ and was subdivided into 100 x 10 μ rectangles. The base line of the grid was laid along the basal pole of the ameloblast (adjacent to the stratum intermedium), thus marking off a 100 μ length of ameloblasts, with successive rectangles overlying these cells. The number of grains in each rectangle was counted until the entire height of the cell was examined.

Similarly, the grains over the area of enamel matrix adjacent to the apex of the ameloblasts were counted by placing the base line of the grid along the edge of the matrix closest to the ameloblasts, and enumerating the grains in progressive $100 \times 10 \mu$ rectangles until the dentino-enamel junction was reached.

Average grain counts were calculated on the basis of a 500μ length of enamel organ, that is, a maximum of five values for each level of cell and matrix, were averaged for each 500μ length of enamel organ. This was possible because it was found that there was usually no variation in either cell height or matrix thickness in any given 500μ length. Within these lengths, however, there were regions which could not be counted. The background fog, determined over tissue-free areas of similar size, were subtracted from each value.

RESULTS

Qualitative observations

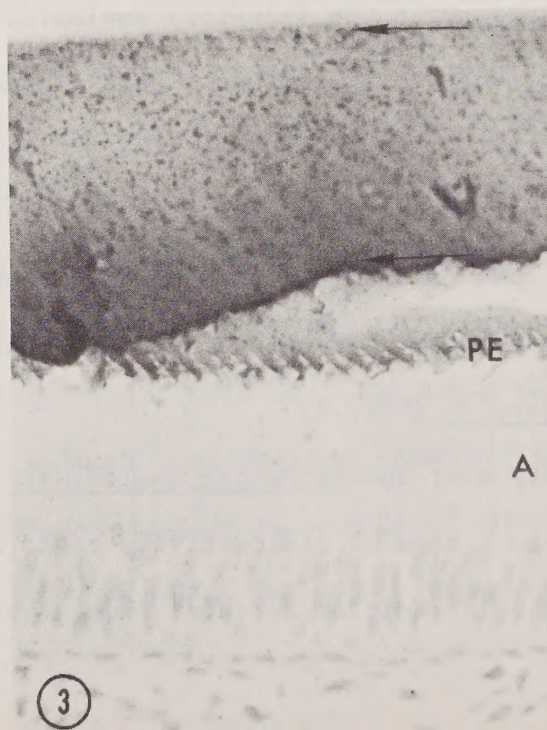
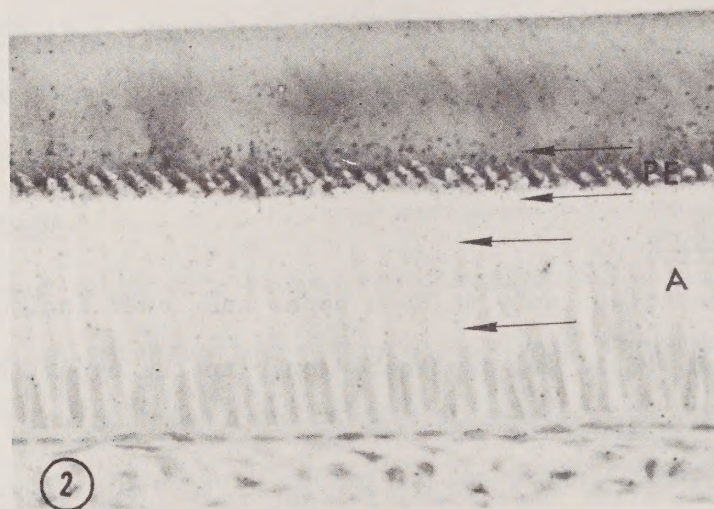
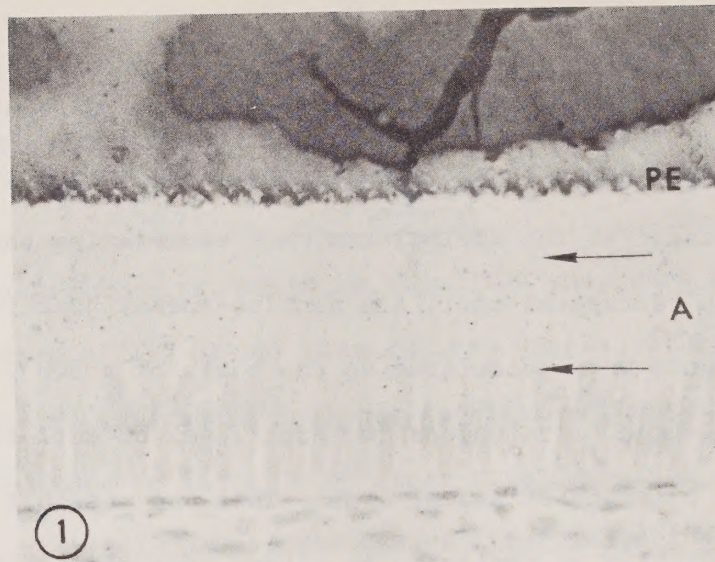
The results given below apply to both upper and lower incisors, but where measurements are given, these are applicable only to the upper incisors.

Five minutes after injection (Fig. 1)

At this early time after injection, a light radioautographic reaction was present over the ameloblasts. Over the secretory ameloblasts, this reaction was not uniformly distributed, but was present as a band of radioactivity mainly over a $10\text{-}15 \mu$ region of cytoplasm beginning 10μ apical to the nucleus and extending to below the apical terminal bars. The nucleus, basal region of the cell (mitochondrial region), and the cell apex (including Tomes process) contained little or no radioactivity. The matrix showed no reactions over it, other than background fog.

Fifteen minutes after injection (Fig. 2)

The reaction intensity over the secretory ameloblasts had increased considerably at this time interval. A band of radioactivity could^{again} be seen over the supra-nuclear region of the cell. In addition to this, another band of intense radio-



activity was seen over the apices of the cells, Tomes processes and over a thin layer of enamel matrix (preenamel). This second band was seen only in relation to the secretory ameloblasts near the formative end of the incisor. The two bands of radioactivity were separated by a layer (10-15 μ wide) of relatively non-labelled cytoplasm.

One hour after injection

The two bands of radioactivity could be indistinctly recognized over the secretory ameloblasts, although the supranuclear band was somewhat diffuse. A light reaction still persisted over the nucleus and the base of the cell.

The reactions over the enamel matrix extended from the formative end incisally to the region of the regressing ameloblasts, a distance of about 6 mm from the formative end of the tooth. At the formative end, where the layer of enamel matrix is very thin, the entire matrix was heavily labelled; as the thickness of the matrix increased, the band of radioactivity broadened, but was only 20 μ in thickness, while the entire enamel matrix was 80 μ thick. This was seen at about 4 mm from the formative end. At 6 mm, the band of radioactivity had decreased to a width of 10 μ , and then it completely disappeared. On the dantinal side of this radioactive band a decreasing gradient of radioactivity could be found in the region of secretory ameloblasts, but towards the regressing ameloblasts, the matrical reaction was confined to the band of radioactivity mentioned above.

Twelve hours after injection

At this time interval the ameloblasts showed a relatively intense reaction. This was still localized to a diffuse supranuclear band. The matrical reaction was very intense at the extreme formative end, where the entire thickness of it was labelled. A band of intense radioactivity, 20 μ wide, was about 20 μ distant from the apices of the secretory ameloblasts, then sloped gradually down to the apices of the regressing ameloblasts at about 3 mm from the formative end. The diffuse

gradient of radioactivity on the dentinal side of this band had considerably increased in intensity in the region of the secretory ameloblasts, whereas it completely disappeared in the region of the regressing ameloblasts, that is, at about 4 to 5 mm from the formative end of the incisor. A diffuse, but more intense gradient of radioactivity was also seen between the cell apices and the heavily radioactive band in the matrix. This was seen where the band was at a distance from the cells.

Four days after injection (Fig. 3)

The reaction over the ameloblasts has, by now, decreased in intensity. However, the remaining radioactivity was still confined to the supranuclear region of cytoplasm.

The band of radioactivity over the enamel matrix, seen at the 12-hour interval, was still present at this time, but now in the region of the secretory ameloblasts. This band was situated almost at the dentino-enamel junction, that is, at 80 μ from the apices of the cells. (No data is available about the ameloblasts and enamel matrix at the formative end because the plane of section through these incisors did not pass through that region.) As the band of radioactivity was followed incisally, it was seen to slope gradually towards the regressing ameloblasts and finally reached the apices of these cells at a distance of about 5.5 mm from the formative end of the tooth. The diffuse gradient of radioactivity on both sides of this heavier band was still high, but was now almost equal on both sides of it, however, when the heavy band was in contact with the apices of the regressing ameloblasts, there was no diffuse reaction between the band and the dentino-enamel junction.

Quantitative results

The extensive qualitative data obtained are too complicated to be given in full in this report (~~see Discussion~~). The greatly simplified table 1 shows the average number of silver grains per 100 μ^2 area of secretory ameloblasts and enamel

matrix. It may be seen that the counts over the various regions of the ameloblasts, from the base to apex, is low at 5 minutes, increases at 15 minutes and 1 hour, shows little change at 12 hours, and is considerably reduced at 4 days. However, the counts of the preenamel and mature enamel show that at 5 minutes no labelling is present over these regions; at 15 minutes, a low degree of labelling is present over the preenamel, but no reactions are seen over the mature enamel. By 1 hour, the reaction is intense over the preenamel, but still very low over the mature enamel. At 12 hours, the preenamel is heavily labelled, and reactions are present over mature enamel matrix. Four days after injection, the mature enamel matrix is most heavily labelled.

Figures 4 and 5 were drawn to represent the positions of the maximum grain concentrations over the ameloblasts and the enamel matrix at two time intervals after injection, that is, 5 minutes (Fig. 4) and 12 hours (Fig. 5). Each of the two graphs represents the counts, the height of the cells and enamel matrix of a single longitudinal sagittal section of an upper incisor from an animal sacrificed at each time interval. The lower graph of each figure represents the height of the cell plotted against the distance in microns from the formative end of the tooth. Similarly, the upper graph of each figure is the thickness of the enamel matrix also plotted against the distance in microns from the formative end. It must be pointed out, that the first figures to the left of each graph does not necessarily indicate the extreme formative end, but it is the region of ameloblasts and matrix nearest that end in that particular section of the incisor.

The solid bars in the cells at 5 minutes (Fig. 4), and in the cells and matrix at 12 hours (Fig. 5) represent the position relative to cells and matrix of the highest concentration of radioactivity as an average over a length of 500 μ . At 5 minutes (Fig. 4) it can be seen that no reactions are present over the matrix, but a band of maximum radioactivity is present over the ameloblasts in a position which

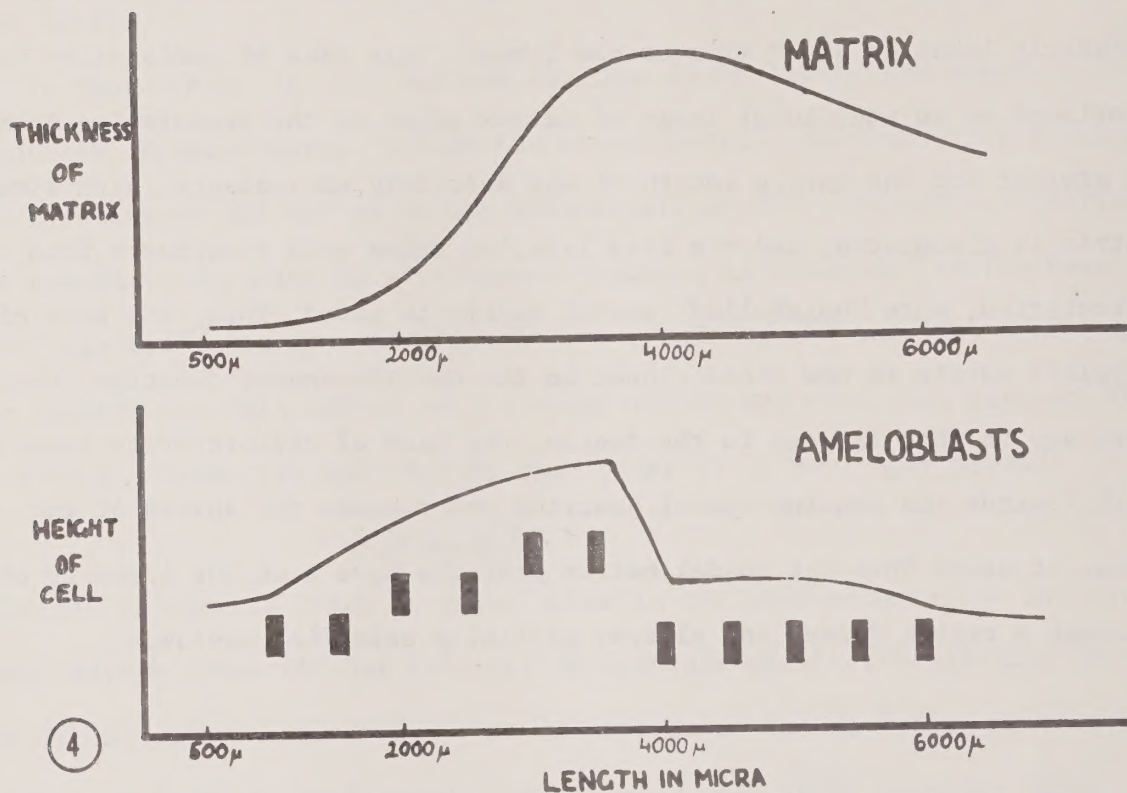
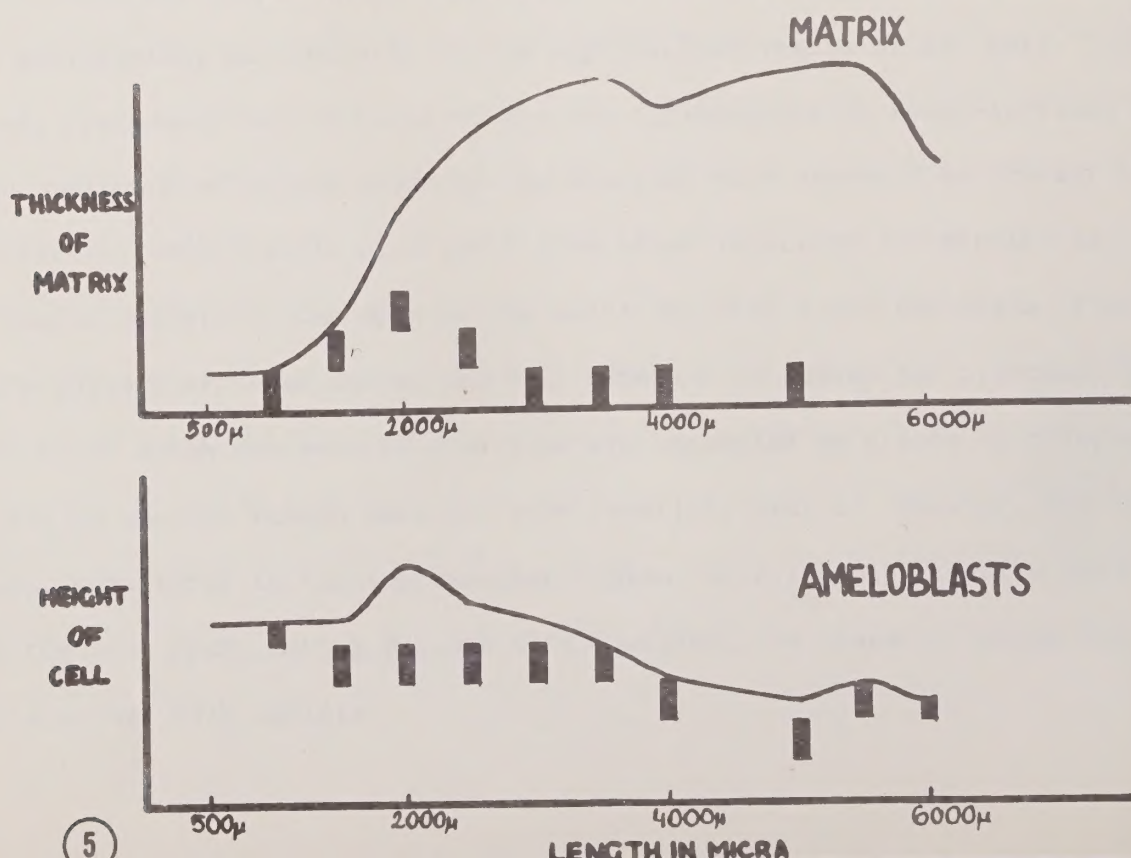
is supranuclear (the nuclei being located between the base of the cell and the reaction band).

At 12 hours (Fig. 5), the maximum reaction intensity in the ameloblasts is still located supranuclearly. Within the enamel matrix, the band of radioactivity is seen to start at the apices of the ameloblasts at 500 μ from the formative end. It then extends towards the dentino-enamel junction and reaches its farthest distance from the cells at about 2000 μ from the formative end. The band then extends towards the cell apices and is again in contact with them between 3000 and 5000 μ from the formative end. Beyond that point it is no longer present.

DISCUSSION

The use of various labelled amino acids in the radioautographic investigations of enamel matrix formation has provided an accurate qualitative picture of this process. Thus, using amino acids commonly present in enamel matrix protein, such as methionine, leucine and proline, and an amino acid least commonly found, tryptophane, a substantially similar pattern of the elaboration and secretion of enamel matrix protein was found. In all cases the proteins are elaborated in the cytoplasm of the ameloblasts, particularly in the supranuclear region of the cell. This synthesis presumably occurs on or within the ergastoplasm or rough-surfaced endoplasmic reticulum which was shown by the electron microscope to be present in this region (Reith, 1960, 1961). At a later time after injection (30 minutes to 1 hour), the proteins migrate to the apex of the cells and into Tomes processes. From here they are rapidly excreted across the cell membrane to become the preenamel matrix. The two bands which are seen at this time are separated by a zone of cytoplasm which for an unknown reason does not show reactions over it. However, the labelled proteins, seen first in the supranuclear region, must rapidly traverse this zone to attain the cell apex. Having reached this position, the transfer across the cell membrane occurs very rapidly.

Concerning the labelled matrix, it may be seen that at the extreme formative end of the tooth, where the matrix is very thin, the entire breadth of it is intensely labelled at 30 minutes and 1 hour. This band of radioactive protein is continued as an additional layer of matrix added to the preexisting enamel, and is present for the entire length of the secretory ameloblasts. With time, as more matrix is elaborated, and the free labelled amino acid disappears from the circulation, more "unlabelled" enamel matrix is added. Thus, the band of heavily labelled matrix is now found closer to the dentino-enamel junction. However, contrary to what is seen in the dentin, the band of radioactivity seems to diffuse both towards the dentino-enamel junction and towards the apices of the ameloblasts. Thus, it seems that the enamel matrix proteins have a unique property of diffusing through a rather dense, and already partially calcified medium.

5 MIN. AFTER TRYPTOPHANE- H^3 UPPER INCISOR12 HOURS AFTER TRYPTOPHANE- H^3 UPPER INCISOR

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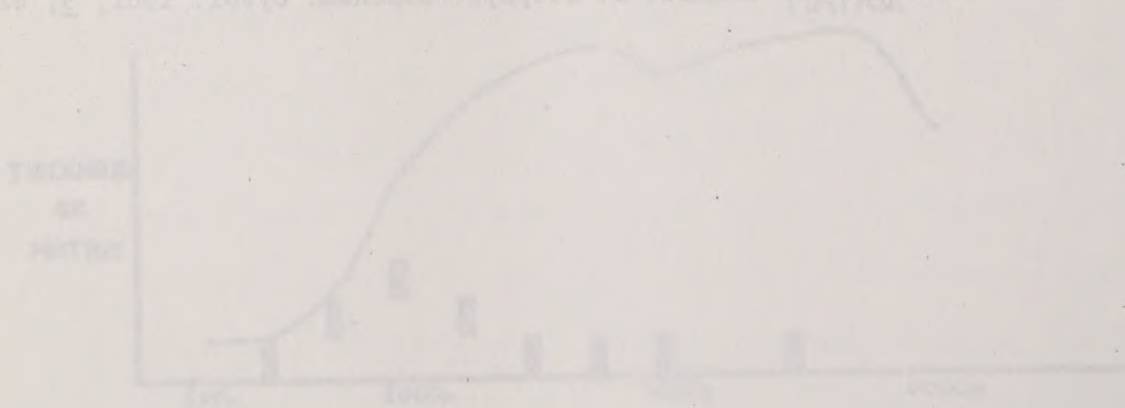
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TABLE 1

AVERAGE NUMBER OF GRAINS PER 100 μ^2 AREA

OF SECRETORY AMELOBLASTS AND MATRIX

Time after injection	Secretory ameloblasts					Matrix	
	Base	→ Apex				Preenamel	Mature enamel
5 minutes	0	2	<u>6</u>	2	1	0	0
15 minutes	1	6	<u>20</u>	10	10	2	0
1 hour	3	6	13	10	<u>20</u>	40	3
12 hours	3	6	8	11	<u>15</u>	50	12
4 days	2		3		3	14	30



LEGEND OF FIGURES

Fig. 1 Radioautograph of the upper incisor of the rat 5 minutes after injection of tryptophane- H^3 . (Sagittal longitudinal section, H & E, x 580, 97-day exposure).

The most intense reaction is seen over the cytoplasm of the secretory ameloblasts (A). The reactions form a band extending from the apical surface of the nuclei to a region somewhat removed from the apical terminal bars (between the arrows). Few reactions are present over the nuclei and the base of the cells. The preenamel (PE), enamel matrix proper shows no reaction (other than background fog). The space between the preenamel and the cell apices is due to artificial separation.

Fig. 2 Radioautograph of the upper incisor of the rat 15 minutes after injection of tryptophane- H^3 . (Sagittal longitudinal section, H & E, x 580, 97-day exposure).

The band of radioautographic reactions over the secretory ameloblasts (A) is localized to the supranuclear region of the cells (between lower two arrows). The nuclei and cell base still show a weak reaction.

A second band of radioactivity is now present over the cell apices and the preenamel (PE), between the upper set of arrows. The scattered grains over the enamel matrix proper is due mainly to background fog.

Fig. 3 Radioautograph of the upper incisor of rat 4 days after injection of tryptophane- H^3 . (Sagittal longitudinal section, H & E, x 580, 97-day exposure).

The reaction intensity over the secretory ameloblasts (A) is considerably reduced. The supranuclear reaction band is now diffuse and recognizable only by grain counts. The preenamel matrix (PE) is not radioactive, however, the matrix proper now shows a diffuse band of radioactivity indicated between the two arrows, where the reaction is seen to extend up to the dentino-

Fig. 3 (cont.) enamel junction. The portion of the enamel matrix proper between the lower arrow and the preenamel (PE) cell apex junction is only diffusely and weakly labelled.

Figs. 4 and 5 Graphs of the position of the maximum concentration of silver grains over the ameloblasts and the enamel matrix of a longitudinal section of the upper incisor at 5 minutes (Fig. 4) and 12 hours (Fig. 5) after injection of tryptophane- H^3 .

The lower graph of each figure shows the height of the ameloblasts plotted against the length of the incisor starting from the first ameloblasts and matrix seen closest to the formative end and extending to the region of the incisor where the enamel matrix is completely lost.

The upper graph of each figure shows the thickness of the enamel matrix similarly plotted against the length of the same incisor.

At 5 minutes (Fig. 4), the band of radioactivity is seen to extend across the cytoplasm of the ameloblasts. No reactions are present over the enamel matrix.

By 12 hours (Fig. 5), the band of radioactivity persists in the cytoplasm of the ameloblasts. Also, a band of reactions is present over the enamel matrix.

NATIONAL RESEARCH COUNCIL, ASSOCIATE COMMITTEE ON DENTAL RESEARCHD.A. 70SUMMARYDecember 21, 1962.

J. P. Lussier

In vitro studies were undertaken to evaluate the effect of different doses of vitamin D on the oxygen consumption by bone chips. At the same time the importance of marrow cells was assessed under the same conditions. Mature bone tissue showed an increase in O_2 uptake under the influence of large doses of vitamin D. In order to bring up this evidence, the animals were tube fed with vitamin D 24 hours prior to sacrifice. Magnesium deficiency which was expected to lower metabolic reactions did not bring the expected results.

The effect of introducing citrate to 2% in the diet on the citrate content in urine, blood and bone was studied in 28 male rats. Citrate was recovered in urine and its concentration was found to be more than twice the normal value. There was no detectable changes in blood and bone.

Further data are reported concerning the project D. T. 70 on the effect of hepatectomy, nephrectomy and of both operations in male rats which were injected with radiocitric acid. The citrate specific activity was determined in blood serum and bone. This step was made possible through some improvements in the analytical methods of radioactive citrate.

INTRODUCTION:

The research project submitted last March and accepted for sponsorship by the N.R.C. Associate Committee on Dental Research under the designation DA 70 had for objectives in vitro studies on bone tissue of animals treated with various levels of vitamin D, and parathyroid extracts. These treatments could be combined with magnesium deficiency nephrectomy and / or hepatectomy.

On account of the time lag unavoidable in the appropriation of the necessary equipment and the familiarization with the delicate techniques of tissue respiration, it was not until late last summer that tangible results could be registered.

In the meantime, a collateral project was carried with the aid of a junior year student. Also further work has been proceeded along with the previous project DT 70. Therefore, the present report includes data from three studies.

- A- in vitro studies in bone of animals treated with vitamin D and / or deficient in magnesium.
- B- the effect of a diet containing 2% sodium citrate on the citrate content of urine, blood and bone.
- C- the specific activity of citrate in bone and blood serum of hepatectomized and / or nephectomized rats.

PART A

The submitted protocols in DA 70 application included a series of in vitro studies to be pursued in four steps:

- 1- Studies on bones of animals treated with various amounts of vitamin D.
- 2- Studies on bones of animals treated with parathyroid extracts.
- 3- Studies on bones of magnesium deficient animals.
- 4- Studies on bones of nephrectomized and / or hepatectomized.

Attention has been less concentrated on part two on account of the difficulty to select at the first phases of these experiments the type of extracts which would be more suitable. This part will be considered at the end of the project.

Previous works have clearly indicated that vitamin D is influencing the oxidative processes in cells responsible for bone growth, namely the cartilaginous layers of the epiphyseal plate. (Tulpule)¹, (Kikshit)², (Picard)³, (Raiha)⁴. On the other hand (Harris)⁵ (Lussier)⁶ have demonstrated how large amounts of vitamin D could promote hyperossification in the growing ends of long bones. This effect was very strongly enhanced when animals were also deprived of magnesium in their diet. It was therefore advisable to establish if these changes were related in some way to the aerobic mechanism of the respiration with special reference to the synthesis and utilization of citrate in bone.

Methods.

The principles advocated by Umbreit et al, (7)

for calibration and operation have been applied. No description of the techniques is necessary except for a specification of the conditions followed. These are summarized in Table I.

In order to maintain as close a relationship as possible with the work we have previously done, we decided to use animals of same strain, age and weight, and to make measurements on the same bones or same parts thereof. The hind limbs were still used to supply the bone tissue. Assays were made to see if a noticeable difference existed between the tibia and the femur. It was indicated in Graph I that some difference existed with respect to the amount of O_2 used per milligram of tissue. After one hour of respiration in a closed O_2 atmosphere, the femur had used 0.19 ul/mg, whereas the tibia had taken only 0.14. After two hours a similar difference still persisted. The femur had used 0.27 and the tibia 0.20. The difference between these bones is 0.035 ul/mg or 15% percentagewise in favor of the femur. However it was soon found that by using only one bone, especially in small treated animals the amount of material was rather low. It was then decided to use one femur and one tibia for each assay.

The importance of bone marrow.

As it was not possible to remove the marrow completely from a long bone such as the femur or the tibia, it was found more practical to use the bone as a whole. In order to assess the part played by the marrow cells in the actual determinations, a series of assays were made to establish a relationship based on the marrow weight and the total bone weight. It was shown that bones (tibia and femur) when they reach a wet weight of about 400 mg, the ratio is one mg of marrow to 11.4 mg of whole bone, and when the wet

weight of both bones reaches 800 mg, the ratio is one mg of marrow to 14.2 mg of whole bone.

As the determination of O_2 consumption were made during two hours, a question rose as to what were the variations due to the marrow proper all along the experiments. Graph 2 shows that during the first hour the values for the marrow respiration were quite stable. After the first hour the rate decreased by 50% within the next hour. This was not found to be parallel to what occurred in bone. Therefore, in all cases control experiments have been carried with marrow alone and values were recorded at the same intervals.

Marrow and Spleen.

It was also thought that due to morphologic and functional similarity of spleen tissue and bone marrow it would be advisable to compare samples of both tissues and bone marrow it would be advisable to compare samples of both tissues under the same conditions. Graph 3 shows that the bone marrow in the young rat has the same respiratory coefficient, although it aged much more rapidly than the spleen. Further work is necessary to know if spleen tissue can be used for control measures of the hemopoietic cells activity. It seems however that damage to the marrow cells during the removal from the bone cavity or the arrangements of cells within the marrow made O_2 less accessible as compared to what was found for the spleen tissue which was prepared in thin slices.

Effect of high dosage of Vitamin D.

Animals received by tube feeding amounts of

vitamin D 3 varying from 50 to 150000 int. units, 24 hours prior to sacrifice. Histogram 1 shows the O_2 consumption in the different assays.

The data on the whole bone showed inconsistent changes. It appeared that some progression took place in the rate of O_2 consumption with the increase in the doses of vitamin D from low to very high levels provided corrections were made to remove the concealing effect of bone marrow tissue.

The calculations were made in the following way

$$\frac{K_b \times N_b}{W_b} - \frac{K_m \times N_m \times R}{W_m} = X \text{ ul}/O_2/\text{hr} / \text{mg bone tissue.}$$

when K_b = constant of manometer and flask containing bone tissue.

K_m = constant of manometer and flask containing marrow sample.

N_b = amount of O_2 used as read on manometer b.

N_m = " " " " " " " " m.

W_b = weight of marrow sample in mgs.

W_m = " " bone sample in mgs.

R = ratio of $\frac{W_m}{W_b}$

W_b

Effect of magnesium deficiency.

Preliminary attempts to put forward the role played by magnesium in the oxidative reactions which are taking place in bone tissue are reported. It is known that Mg is an important cofactor in the activation of several enzymatic systems of the anaerobic respiratory cycle and on the TPN activation.

On the basis that magnesium deficient animals have a low magnesium and a lower content of magnesium in their bones it was thought that this depletion could affect the oxygen consumption in the bone and also affect the utilization of citrate. It was decided to measure both O_2 consumption and the amount of radiocarbonate evolved from the utilization of citrate by bone chips.

The results included in table II demonstrate that bone tissue is not using less O_2 under the influence of magnesium deficiency but slightly more when the animals received vitamin D at the rate of 500 i.u. per day for 50 days.

The results gathered from the addition of radiocitrate in the medium showed a slight decrease in the rate of utilization. However there were enough variations in the data collected from the magnesium deficient animals to inspire caution in interpreting.

It is required to repeat these experiments sufficiently to enable statistical evaluation. The analysis of individual curves of respiration will lead to better understanding than to rely only on the 60 minutes reading. Finally it is expected to determine the DNA content of each sample in order to establish calculations on a cellular unit basis instead of the mere weight unit basis.

PART B

The effect of increased citrate intake on the citrate content of serum, urine and bone.

This project has been carried during the summer 1962 with the aid of Mr. J. Ambrozaitis a third year dental student.

The effect of dietary factors in the citrate production and excretion has already been investigated by some authors. Calcium and phosphate have been selected as parameters by most of them. The ability of citrate taken orally to bring changes on the level of circulating citrate in the citrate storage in bone in relation to the amounts taken and excreted has been incompletely surveyed.

Methods

The following experiment was designed. Male albino rats weighing approximately a hundred grams were fed a diet. Containing 2% of its weight as citrate (Table III). Calcium citrate was selected as the source of citrate as it could be introduced at the expense of calcium carbonate. Therefore the same amount of calcium was presented in the diet of both control and treated animals.

In a preliminary phase of the experiment sodium citrate was included in the drinking water. This procedure was found unpractical as the amount injected could not be easily controlled. Including citrate in the solid part of diet, although it does not prevent variations in the intake permits a fairer approximation than does inclusion in the water. After a 15 day period, all animals are likely to have consumed the same amount of food especially when there is no undue variations

in the individual body weight increase.

Animals were kept in individual cages fastened to glass funnels and weighed every second day. The food and water supply was kept constant. Urine collections were made every Monday, Wednesday and Friday. After 15 days, animals were sacrificed with ether. Blood was collected immediately. Femurs were excised, dissected, then dried under vacuum at 75°C.

Citrate was determined according to Bauer's modification of Pucher method. Blood was allowed to clot; one ml of TCA 10% was added to one ml of serum. After centrifugation, determination were run in duplicate on two 0.5 ml serum aliquots.

One femur was placed in a test tube with 5 ml of H_2SO_4 20%. The determination was run on 0.5 ml aliquots of this solution.

Urine was collected in beakers containing 5 ml of H_2SO_4 20% to prevent any loss through microbial contamination. The mixture was diluted to 10 ml with distilled water and 0.5 ml aliquots were used for citrate determinations.

Results

The growth curves of both normal and treated animals are shown in graph 4. It is obvious that after the fifth day of the experiment, the citrate fed animals are not tolerating the resulting imbalance without undue effects. The change in the growing rate coincided with an increase in the level of urinary citrate excretion as seen in graph 5. It could be that within the first five days i.e. the period during which the animals are getting adjusted to the increased load of citrate, loss of calcium, sodium or other important cations through

the urine creates a limiting growing factor to remain in subtotal amounts in the body. It could also be that citrate would, already in the gut, present absorption of growing factors in optimum amounts.

The slope of the curve from normal animals is an effect of growth. If the data were expressed as the weight of citrate excreted per 100 grams of body weight, the values would be on an horizontal line. This comment also holds for the treated animals.

Whatever the effect is on the organism in general, the circulating fluids and bone do not seem to be affected at all (Table IV). The regulating factors of citrate concentration are not exceeded under the present conditions, therefore the bone is not. It would be interesting at this stage to study the effect of increasing intake of citrate in the diet to the point where citricemia may become upset and to see what effect on bone it could have thereafter.

It is our intention to vary the form of citrate in order to differentiate more precisely the effects of citrate proper from those of combined cations. Ammonium citrate, sodium citrate and mixtures of these with calcium citrate may bring interesting results.

PART C

On the experimental material collected from the project DT 70, further assays were made which enabled us to supply more data on the effect of nephrectomy and hepatectomy on citrate metabolism as indicated from the citrate specific activity in blood and bone.

Methods:

A technical difficulty was encountered in the determination of radioactive citrate in bone. This had been mentioned in the previous report as causing a serious delay in the collection of data from the above mentioned material.

It is not easy to extract bone citrate from bone. Bone salts including citrate are released from bones when they come in contact with an acid or a low pH buffer solution. However, the resulting bone solutions cannot be readily used for determination of radiocitrate. Citrate should be preferably isolated.

Some difficulties arose on account of the low quantity of total citrate (less than 100 micrograms / ml) and the high amount of strong acid in the bone solution. Table VII shows some of the different methods tried and the results obtained.

After these unsuccessful attempts it was decided on the ground that citrate was the major radioactive compound in the bone solution to oxidize it and to determine the radioactive CO_2 . As a matter of fact there is in bone more than a hundred times more citrate than any other organic salt. The chromatography of bone solutions have revealed that citrate was the only noticeable radioactive compound.

By a simple modification of the Van Slyke's wet combustion method, citrate could be oxidized to such an extent as to release entirely the radioactive carbon contained in the carboxylic group 1 and 5 of the product we have used.

- 1- A 0.1 ml aliquot of bone solution was introduced in the flask with 2 ml of concentrated sulphuric acid.
- 2- The flask was connected to the modified Van Slyke apparatus. From the delivery funnel 2 ml of KMnO_4 1.5 N were poured in the flask.
- 3- The mixture was gently heated to boiling and maintained at this point until no more gases escape through the mercury manometric valve.
- 4- The ion chamber was removed from the Van Slyke apparatus and placed on the converter for the measurement.

Calculations:

Up to the time, this ion chamber method was used, the results were expressed in terms of counts per minute and determined with the GM Counter. With the use of the new method, a conversion or equivalence factor was established so that the number of amperes could be easily transposed into counts per minute or vice versa.

Samples of a solution of pure radioactive citric acid were used on both apparatus and from the respective readings the following relationship was established. (Graph. IX).

The determination of serum citrate and specific activity was made with the Geiger conventional method. Half a volume of solution of trichloroacetic acid 20% was added to a volume of serum contained in well stoppered centrifuge tube and joined with a glass tube to a vial containing Na OH 2 N. TCA at the same time it

precipitated serum proteins released CO_2 which was entrapped in NaOH. After heating the mixture in the centrifuge tube to boiling, the tube was centrifuged and the supernatant plated on a planchet evaporated and counted under a thin window GM Counter. An aliquot of the NaOH solution with radioactive CO_2 was similarly treated and counted. The values were plotted in graphs 7 and 8.

Total serum citrate was determined according to Bauer's modification of Paucher Method on serum pools from at least three animals from each subgroup. These data are summarized in table VI.

Results:

A- The specific activity of serum citrate

The effect of hepatectomy on the specific activity of citrate in the blood serum has only a transitory effect within the first two hours. No remarkable difference persists at the end of the second hour. This holds for the specific activity itself as well as for the total radioactivity found in the serum- TCA filtrate as no difference exists between the serum citrate concentration. The values found were all close to 5.5 mgms per hundred ml of serum.

The nephrectomized animals at the third and fourth hours following the injection of radiocitrate had still blood serum with a high content of radioactive compounds. However on account of the hypercitricemia (over 10 mgs %) the specific activity was somewhat higher than the normals or hepatectomized but the rate of disappearance suggested that utilization is taking place at a much slower pace. i.e. about 50% or less than the normals.

The hepatectomized-nephrectomized rats had a blood serum content in radioactive compound which remained constant until the fourth hour when these values were divided by their respective

citricemia the values for citrate specific activity become quite irregular but they remained far above whatever was found in previous groups. Therefore, in the hepatectomized-nephrectomized animals almost no citrate metabolism seemed to take place. The data as they are reported now have not been corrected for the delaying effect of the sluggish circulation in the kidney deprived animals.

The relatively low value found in the NH animals four hours following the injection seems quite unreactistic. It will have to be reassessed by repeating this part of the experiment.

The specific activity of bone citrate.

It is shown in graph 6 that the specific activity of bone citrate is quite comparable in normals hepatectomized and even in nephrectomized animals. With the exception of the value on the first hour about the nephrectomized animals, bone is undergoing a slow but consistent desorption of exchanged radiocitrate. In the case of HN animals the slope is identical but on account of the high citricemia the adsorption has been greater. Thus it took a longer time to get rid of the radiocitrate. However this appears to be the reflection of a passive phenomenon strictly under the dependence of the citrate blood level.

It would be interesting now to see how would react the HN animals when injected with smaller amount of radiocitrate or other citrate precursor following pretreatment with parathyroid hormone or vitamin D.

The most surprising effect to admit from a first glance at Graph 7 would be the relatively low level of radioactive carbon in serum bicarbonate as compared to the absolute amount of radioactive citrate found in serum. It is true that our lack of concern for the RBC might have taken away a large proportion of the blood radiocarbon. The remaining radioactive CO_2 is so low as to render the determinations much less reliable than for citrate. The fact that blood had not been collected under oil and that coagulation took place in open air was enough to produce a substantial loss of CO_2 into the ambient atmosphere.

Nevertheless, there is some plausibility that the serum CO_2 might have behaved as such in the case of hepatectomized and nephrectomized animals. However it is quite improbable that values in serum CO_2 from HN animals might have been maintained that high when on account of a very low metabolic rate citrate values found in blood as well as the bone and respiratory gases were indicative of the contrary.

Therefore, the specific activity of blood CO_2 will not be taken into account in the final report.

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TABLE I

Conditions concerning reagents and experimental procedures (1)

Apparatus = Bronwill UV model equipped with standard 13 ml
reaction vessels.

Medium: Krebs - Ringer Solution with phosphate buffer.

Special reagent: Radioactive citric acid = 0.2 ml of 5.0 mM solution.

CO₂ absorbent = Na OH 5%.

Shaking rate = 100 excursions per minute.

Reading time = 10 minutes.

Total duration = 2 hours.

Selected time for comparison = one hour.

Bone weight (wet): approximately 800 mgms.

Marrow " " : 40 - 70 mgms.

TABLE II

O₂ consumption of magnesium deficient animalsEffect of hypervitaminosis D. concurrently
carried with the magnesium deficiency.

Magnesium def. without vit. D.	N	CO ₂ (ul / mg / hr.)			N	Radioactivity found in CO ₂ as % of citrate added per % mgs of B.M. 0.87
		Whole Bone	Marrow	Bone tissue		
	4	0.251	1.22	0.135	3	
Magnesium def. with vit. D.	2	0.244	1.37	0.154		
Normal controls	2	0.250	1.24	0.128	2	1.14

TABLE III

<u>Diet with added citrate</u>	<u>Control diet</u>
Whole wheat	30.3 %
Casein	16.6 %
Skim milk	8.3 %
Milk powder	8.3 %
Alfalfa	12.5 %
Wheat-germ	12.5 %
Yeast	5. %
Calcium Citrate	2.7 %
Iodine salt	1. %
Potassium iodide	0.2 %
Cod-liver oil	3.2 %

2.7 grams of calcium citrate supplies 2 grams of citrate per % grams of diet.

CITRATE IN URINE.

Days on diet		4		6		11		13	
Controls	N	Mean gms %	Rouge Low High	N	Mean gms %	Rouge Low High	N	Mean gms %	Rouge Low High
	4	0.190	.075 - .315	4	0.400	.170 - .532	4	0.710	.190 - 1.670
Citrate treated animals.	N	Mean gms %	Rouge Low High	N	Mean gms %	Rouge Low High	N	Mean gms %	Rouge Low High
	8	0.606	.195 - 1.230	8	1.200	.350 - 1.350	8	0.728	.190 - 1.310

TABLE V

CITRATE CONCENTRATION IN SERUM AND BONE IN RATS

FEED A 2% CITRATE DIET FOR TWO WEEKS

DIET	N	CITRATE IN SERUM		N	CITRATE IN FEMUR	
		Mean mg %	S.D.		Mean mg %	S.D.
With 2% Citrate	20	4.81	+ .87	19	0.278	+ .021
Without Citrate added	6	4.75	+ .31	7	0.280	+ .016

TABLE VI

<u>Groups</u>	<u>Hours after injection of radiocitrate</u>	<u>Citrate content (mgs. % ml serum).</u>
<u>Normals</u>		

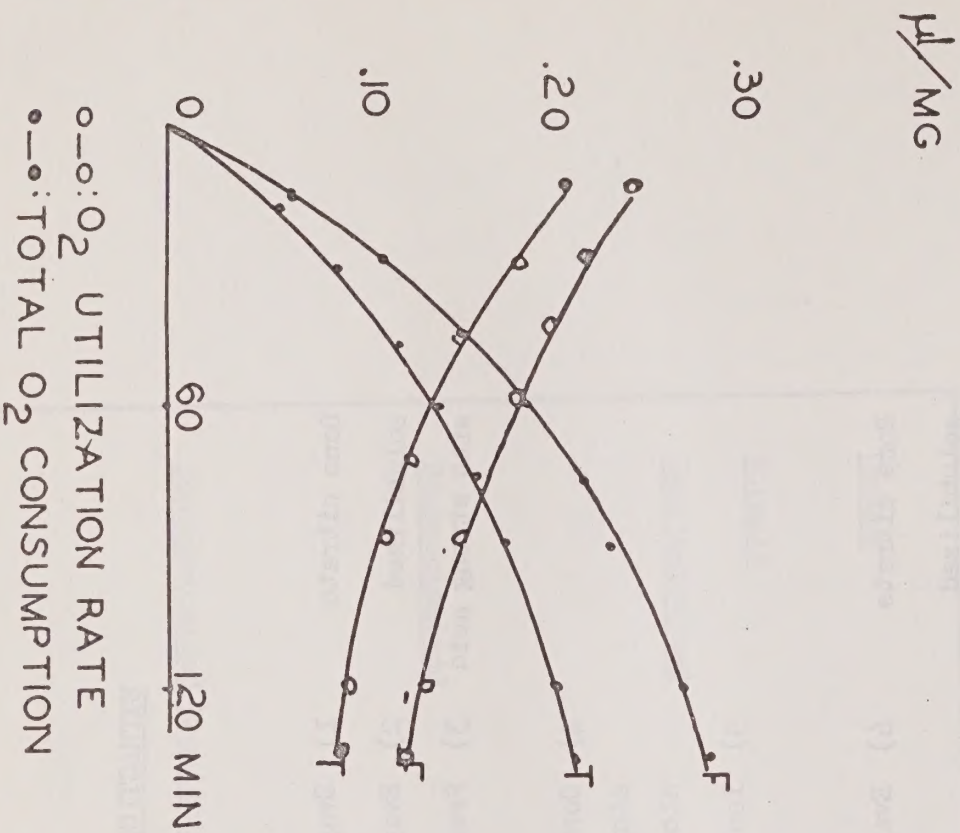
	1	5.76
	2	5.28
	3	5.04
	4	5.51
		6.12
<u>Hepatectomized</u>		
	1	4.56
	2	11.28
	3	10.08
	4	13.68
<u>Nephrectomized</u>		
	1	31.20
	2	26.40
	3	23.80
	4	10.80
<u>Nephrectomized-Hepatectomized</u>		

TABLE VII

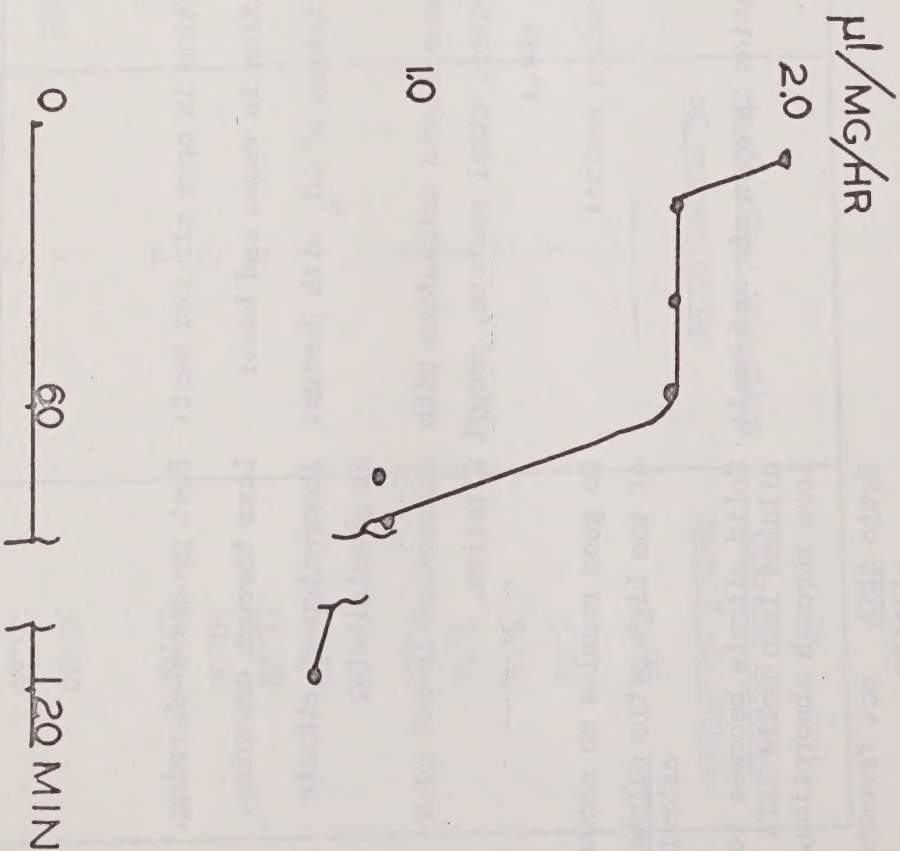
EXTRACTION OF CITRATE FROM BONE SOLUTIONS

Bone citrate solubilized with strong acid	1) Evaporation in open air and heat: 2) Evaporation in vacuo and heat: 3) Precipitation of SO_4 with Barium: 4) Continuous liquid extraction with ethyl ether, ethyl acetate, propyl alcohol, etc.: 5) Ion exchange resins:	Heat produces charring. Loss through creeping. Absorption of citrate in precipitate. Extraction is not quan- titative. No good results on account of too high ratio <u>sulphate</u> citrate.
Bone citrate solubilized with EDTA	6) Evaporation in open air and heat:	Solid citrate becomes too diluted into solid EDTA salts: loss through absorption. Ratio <u>EDTA</u> citrate not favourable to plating. Same as 3.

GRAPH 1: O₂ CONSUMPTION
IN TIBIA & FEMUR OF NORMAL RAT

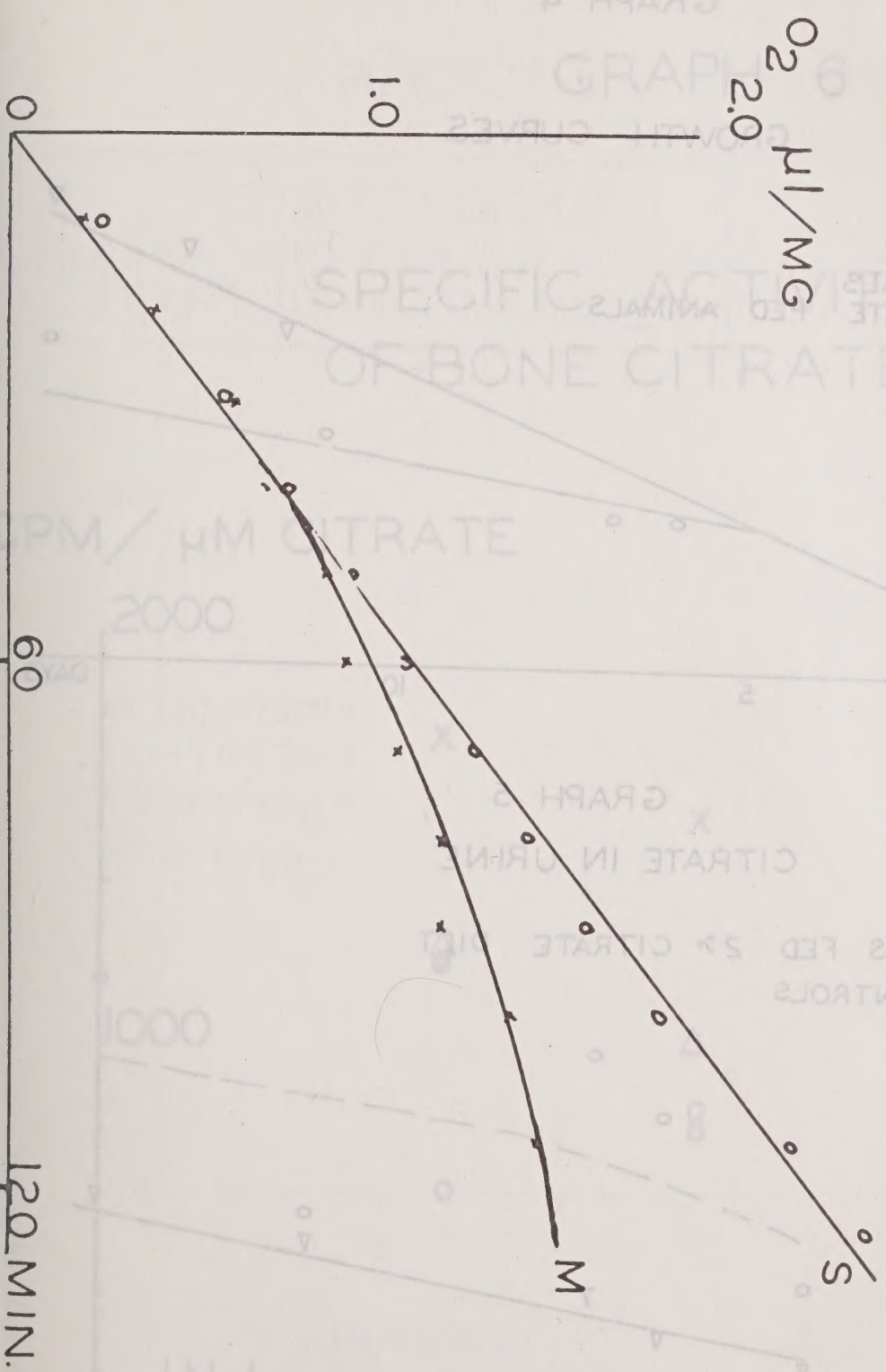


GRAPH 2: O₂ CONSUMPTION
IN BONE MARROW



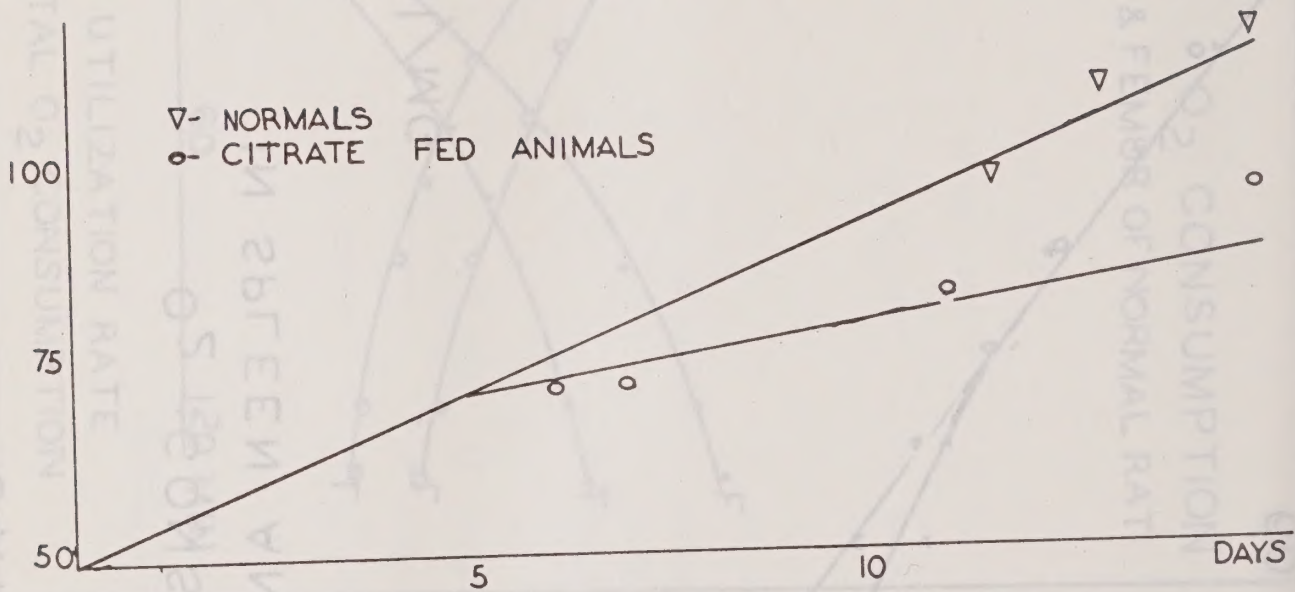
GRAPH 3

O₂ CONSUMPTION
IN SPLEEN AND MARROW



GRAPH 4

GROWTH CURVES

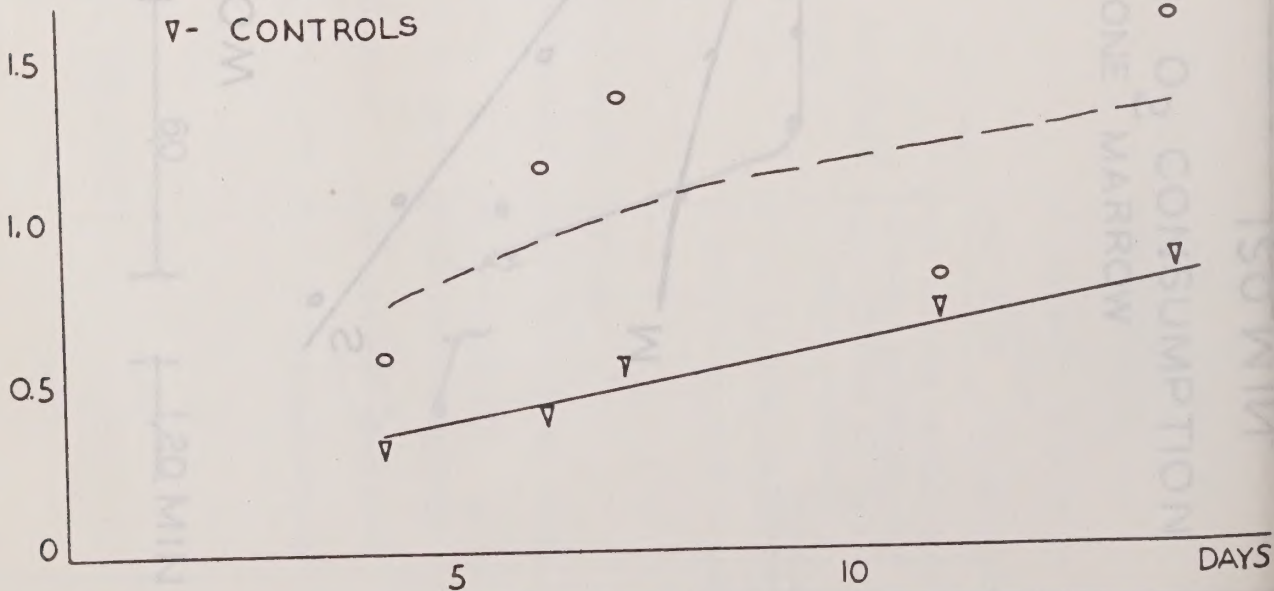


GRAPH 5

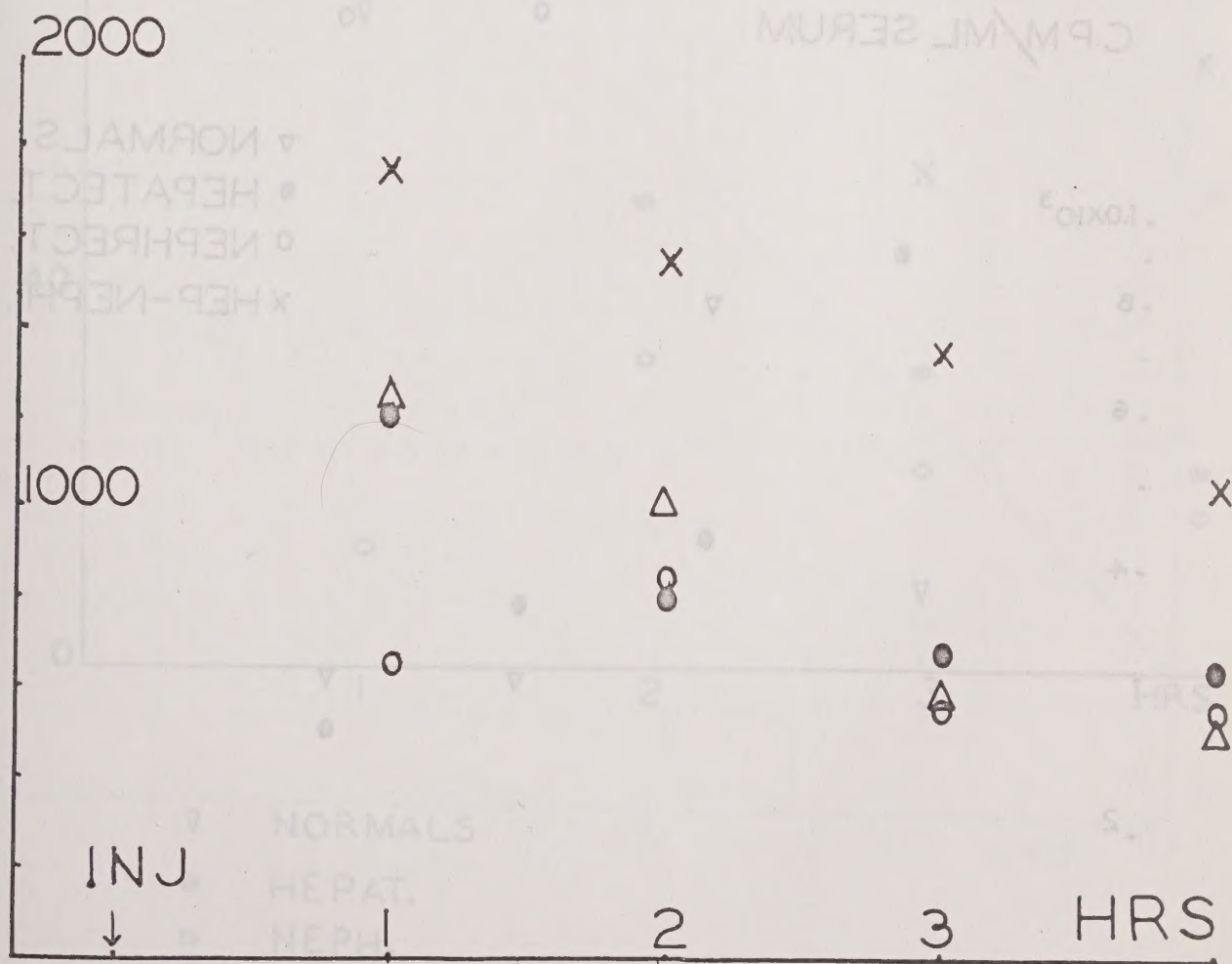
CITRATE IN URINE

CITRATE
(MGS)

○ - RATS FED 2% CITRATE DIET
 ▽ - CONTROLS



GRAPH 6

SPECIFIC ACTIVITY
OF BONE CITRATECPM / μ M CITRATE

GRAPH 7

1.0×10^4

.8

.6

.4

.2

CPM/ML SERUM

RADIOACTIVITY
FOUND IN
SERUM TCA FILTRATE

- ▽ NORMALS
- HEPATECT.
- NEPHRECT.
- x HEP-NEPH.

1.0×10^3

.8

.6

.4

.2

1.0×10^2

HOURS

2

3

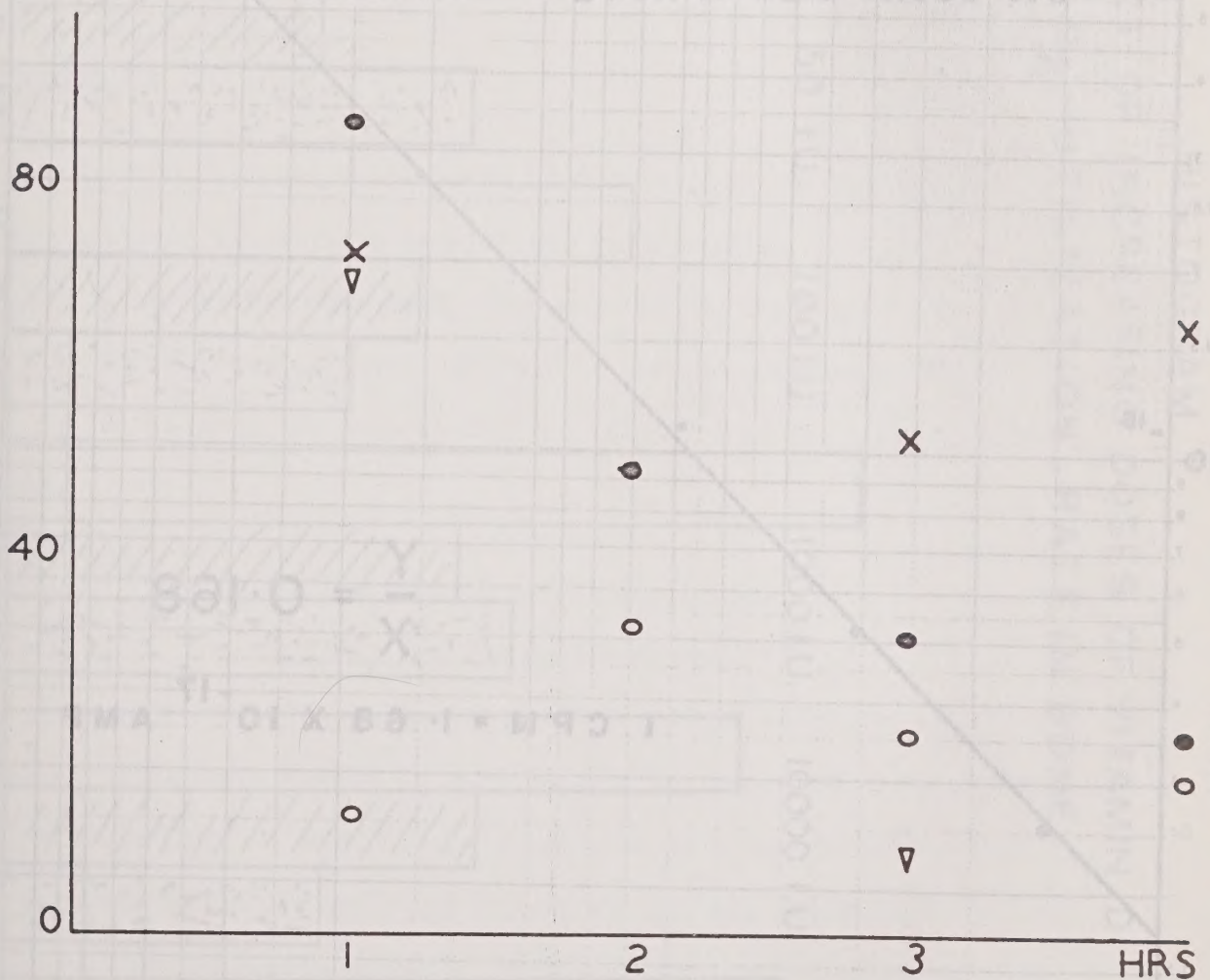
4

1

GRAPH 8

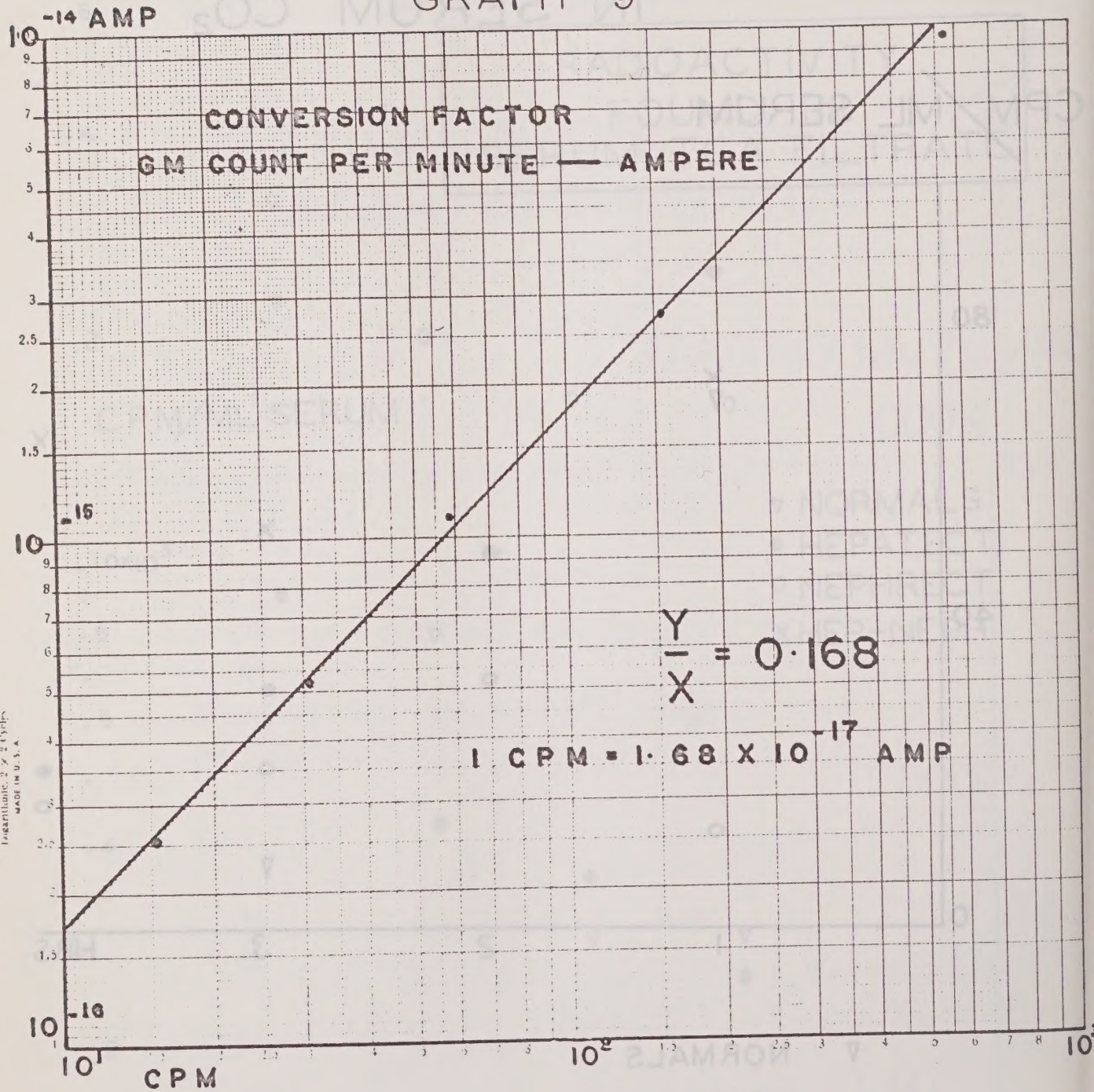
RADIOACTIVITY RECOVERED
IN SERUM CO_2

CPM / ML SERUM

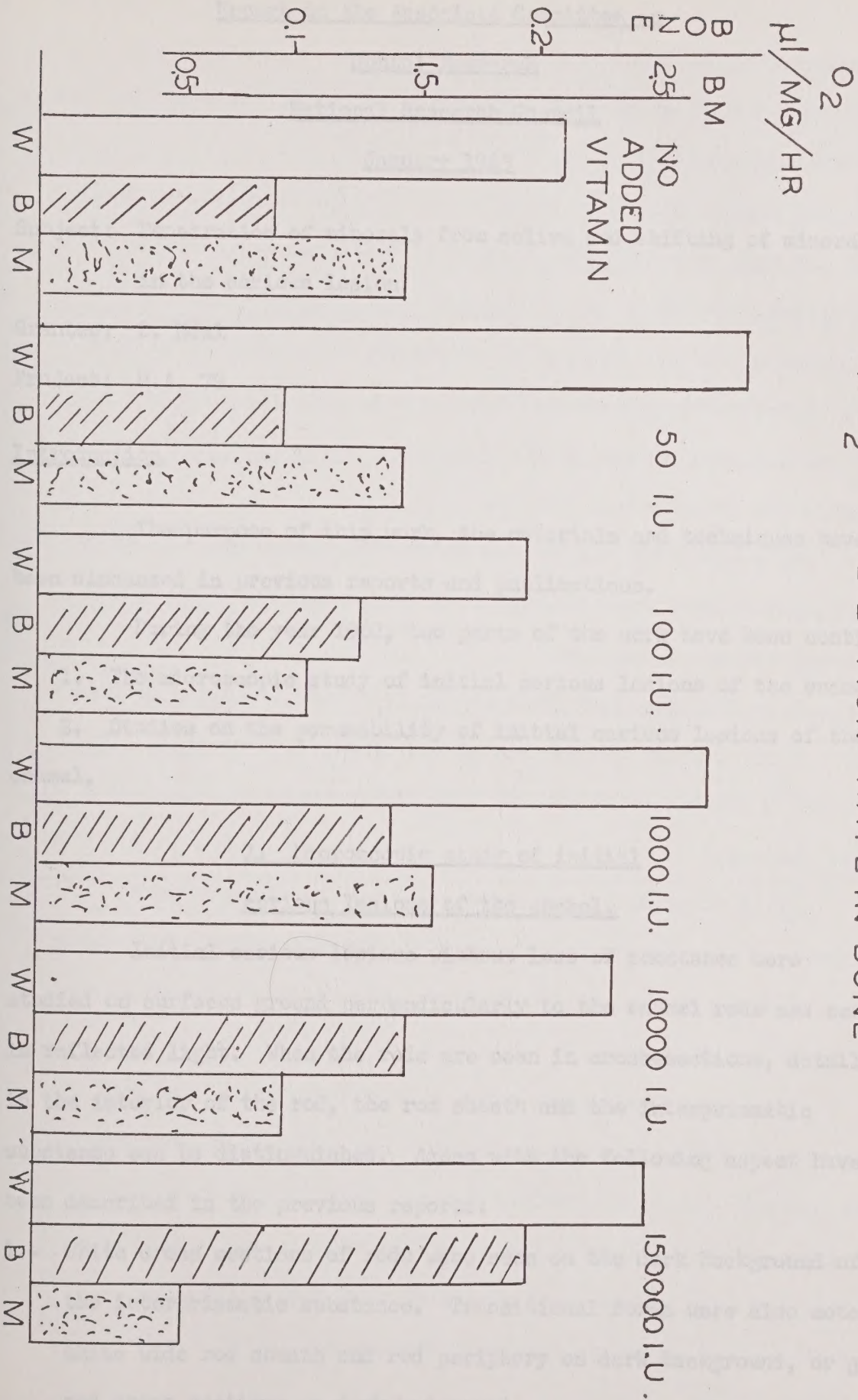


- ▽ NORMALS
- HEPAT.
- NEPH.
- x HEP-NEPH.

GRAPH 9



HISTOGRAM I
EFFECT OF INCREASING DOSES OF VITAMIN D
ON O₂ UTILIZATION RATE IN BONE



Report to the Associate Committee on

Dental Research

National Research Council

January 1963

Subject: Penetration of minerals from saliva and shifting of minerals
in the carious lesion.

Grantee: Z. Mész

Project: D.A. 79

Introduction

The purpose of this work, the materials and techniques have been discussed in previous reports and publications.

During the year 1962, two parts of the work have been continued:

1. The microscopic study of initial carious lesions of the enamel.
2. Studies on the permeability of initial carious lesions of the enamel.

1. Microscopic study of initial
carious lesions of the enamel.

Initial carious lesions without loss of substance were studied on surfaces ground perpendicularly to the enamel rods and seen in reflected light. When the rods are seen in cross-sections, details in the interior of the rod, the rod sheath and the interprismatic substance can be distinguished. Areas with the following aspect have been described in the previous reports:

- 1.- White cross sections of rods were seen on the dark background of the interprismatic substance. Transitional forms were also noted: white wide rod sheath and rod periphery on dark background, or grey rod cross sections on dark background.

- 2.- Homogeneous white highly reflecting areas.
- 3.- Thin white rod sheaths, dark peripheries and white cores of the rods on dark background.
- 4.- Dark rod cross sections on white background and transitional forms such as: rods with dark peripheries and white centers on white background.
- 5.- Homogeneous, cloudy, grey, semitranslucent areas with transitions to a translucent, dark or pigmented aspect.
- 6.- Semitranslucent, cloudy, finely granulated areas with slightly visible greish rod cross sections.

~~Table one shows how many times each of these alterations have been seen in the three main types of lesions in the first 300 cases examined in this project.~~

Over 500 cases have been examined by this technique in this project. Table I shows the results of 500 cases, i.e. how many times each of the 6 described alterations have been seen in the three main types of lesions.

White rod sheaths and white rod cross sections on dark background and white areas are stages of demineralization. Soft white masses form large centers of the lesions, white rods and sheaths form zones on their periphery.

White rod sheaths and cores on dark background have been interpreted as stages of remineralization (by shifting or from outside of the lesion). These alterations are not frequent and form only small areas on the periphery of the lesions which seems to indicate that they are only transitional stages or that some particular circumstances are necessary for their formation.

Table IMicroscopic alterations formedin carious lesions

	Number of observations in:			total
	white lesions	brown lesions	arrested caries	
White rods, dark background	147	90	18	255
White area	154	152	85	391
White cores, dark background	22	38	32	92
Dark rods, white background	42	58	46	146
Semitranslucent, homogeneous	38	130	129	297
Semitranslucent, cloudy structure	122	160	128	410
Number of lesions	181	173	146	500

Translucent and semitranslucent hard homogeneous, white or pigmented areas have been interpreted as remineralization following demineralization and alteration and homogenisation of the organic matrix. They are quite frequent in brown lesions and arrested caries.

Semitranslucent areas with cloudy structure have been named diffuse remineralization. They are very frequent and usually form zones on the periphery of the lesions.

In this part of the work no alterations have been seen which could not be classified in one of the groups described in previous reports.

The lesions were classified according to a classification suggested in 1960. The microscopic classification is based on the presence or absence of areas characterising demineralization or remineralization.

1. Progressive caries shows demineralization only.
2. Alternating caries shows demineralization in some areas and remineralization in other areas.
3. Regressive caries shows areas of remineralization, no evidence of progress of demineralization, i.e. no zones of initial demineralization. Demineralized areas in the center of the lesion are present.
4. Arrested caries, in the sense used here, are lesions that have been entirely remineralized.

Table II shows the microscopic classification of the 500 lesions examined and the relation of the microscopic to the macroscopic aspect.

Table II

Microscopic classification
of initial carious lesions

	White lesions	Brown lesions	Arrested caries	Total
Progressive caries	50			50
Transitions	1			1
Alternating caries	96	74	4	174
Transitions	1	5	1	7
Regressive caries	23	71	62	156
Transitions		3	9	12
Arrested caries	10	20	70	100
Total:	181	173	146	500

The plan was to examine equal numbers of each type of lesions, but not enough arrested caries were available. Even so, dark spots and arrested caries form a considerable part of the studied material while in reality they are only a small fraction of all cases.

Brown lesions are of various types: some are light brown, some are dark; some are pigmented throughout, some only at the surface. At present, it does not seem useful to subdivide them in further groups with respect to the degree of pigmentation.

Arrested caries in clinical sense includes two types of lesions: 1. Lesions, which are remineralized throughout and can be considered healed. 2. Lesions which are microscopically regressive caries with small demineralized areas in deeper parts. They can be considered as inactive but not healed lesions.

A small number of lesions could not be classified, being transitions between two groups.

103	Number of initial carious lesions examined
104	Lesions with a well mineralized superficial zone
105	Lesions with incipient or poorly mineralized superficial zone
106	Lesions without any carious zone at the surface
107	Remineralized lesions of F. 35 type the lesions visible on the radiographic films
108	Remineralized lesions (F. 35 type) visible only on the radiographic films
109	Lesions of F. 35 type the lesions visible on the radiographic films

2. Studies on the
permeability of initial carious lesions
of the enamel

During the year 1962, the penetration of phosphate into initial carious lesions was studied.

The teeth were immersed for 4 hours in a solution of 0.02 calcium hydrogen orthophosphate labeled with P. 32 in distilled water, 1.1 mc in 700 cc.

The penetration of the phosphate into the lesions was visualized by autoradiography. After submersion, the teeth were cut in serial sections on a hard tissue microtome and contact autoradiographs were made. The sections were polished, mounted in distilled water, examined in polarized light and compared with their autoradiographs.

A summary of the results, including the work described in previous reports, is given in table III.

Table III

Number of initial carious lesions examined	261
Lesions with a well mineralized superficial zone	98
Lesions with interrupted or poorly mineralized superficial zone	39
Lesions without any particular zone at the surface	124
Penetration Of P. 32 into the lesion visible on the autoradiographs	154
Penetration faintly visible or in parts of the lesion only	19
No penetration of P. 32 into the lesion visible on the autoradiographs	88

The penetration of phosphate into the lesion is related to the presence or absence of the mineralized superficial zone, as shown in table IV.

Table IV

Mineralized superficial zone present and penetration of P. 32 not visible	81
Mineralized superficial zone present and penetration of P. 32 visible	11
No mineralized zone at the surface and penetration of P. 32 visible	123
Incomplete superficial zone and/or penetration of P. 32	46

In some of the cases in the last group, either the superficial zone or the penetration of P. 32 or both are not clearly visible. In some cases of this group however, the superficial mineralized zone is interrupted and the autoradiographs show penetration of P. 32 only in that part of the lesion where the superficial zone is missing.

In 38 cases, first the penetration of the solution of calcium phosphate from outside into the lesion was examined. Then the uptake of P. 32 in ground sections of the same lesion, submerged in the radioactive solution, was studied. After the first autoradiographs have been made and the penetration determined, the ground sections were submerged in the phosphate solution for 1 hour and second autoradiographs were made.

In 28 cases, the mineralized superficial zone was evident. In 26 of these cases, no penetration of P. 32 was evident on the first autoradiographs and a quite considerable uptake of P. 32 in ground sections was visible on the second autoradiographs. In one case, the first and the second autoradiograph were negative. In one case, both were positive.

In 5 cases, the superficial zone was poorly developed. In 2 of these, no penetration was visible on the first autoradiographs, a high uptake of P. 32 was evident on the second autoradiographs. In 3 cases, both autoradiographs were positive.

In 5 cases, there was no particular zone at the surface of the lesion and in all cases both autoradiographs were positive.

On the second autoradiographs, the lesions appear very dark, although the time of submersion was much shorter. The second autoradiographs show that the carious enamel in the deeper parts of the lesion can take up a considerable quantity of phosphate if this can enter directly, without passing through the mineralized superficial zone.

The penetration of calcium phosphate into artificial lesions produced in vitro as described in the previous report was studied by the same technique.

Lesions produced by lactate buffer solutions: examined 12

abundant penetration of P. 32 12

mineralized superficial zone 6

poorly formed " " 5

no particular " " 1

Lesions produced by acetate buffer solutions: examined 12

abundant penetration of P. 32 12

mineralized superficial zone 5

poorly formed " " 1

no particular " " 6

Discussion

1.

The results of microscopic studies have been discussed in previous reports. Results shown in table I confirm previous conclusions.

The microscopic classification of initial carious lesions of the enamel and the results shown in table II correspond satisfactorily with clinical experience. Progressive caries appear in a small number, because either they soon form a cavity (and cavities were not studied) or they develop into alternating caries. The majority of initial lesions are microscopically alternating or regressive caries. Pigmented lesions are mainly regressive and arrested caries. Only about half of clinically arrested caries are really healed lesions.

The microscopic classification appears to be more exact than clinical evaluation of the lesions. Its usefulness for clinical diagnosis would deserve further investigation.

2.

The studies on the permeability of initial carious lesions performed in simple conditions (neutral aqueous solutions) have shown conspicuous differences: some lesions were readily permeable, some did not show any appreciable penetration after a considerable time.

Table IV and the previous report shows that a well developed superficial mineralized zone did not allow the penetration of solutions into certain lesions while lesions without such a zone were permeable.

Table IV and the study of artificial lesions show, that there are some differences in the properties of the superficial zone in the cases where it appears microscopically well developed. In 11 of the 98 cases

of caries and in all artificial lesions the zone was permeable (and similar results with stains were shown in the previous report.)

It is obvious that a progressing carious lesion must be permeable. It could not deepen if the acid could not penetrate inside and if the minerals could not be dissolved out. To be arrested, the lesion must allow the penetration of minerals from outside.

Hence the impermeability of certain carious lesions appears to be a temporary state. Its duration is obviously important for the evolution of the lesion.

What produces the impermeability and what removes it?

These questions deserve further investigation for which a project is submitted for 1963.

Résumé and conclusions.

A technique has been developed for the microscopic examination of surfaces ground and polished perpendicularly to the enamel rods and seen in oblique reflected light. This technique shows with fine structural details the distribution of minerals in the rod cross sections, rod sheaths and interrod substance.

Over 500 initial carious lesions of the enamel have been studied by this technique. Alterations produced by caries have been described, explained and classified.

Demineralization progressing from rod sheaths and rod peripheries to entire rod cross sections and interrod substance occurs mainly in initial white lesions, it is associated with gradual softening and has been reproduced by acid demineralization in vitro.

Alterations interpreted as remineralization (either by a shifting or by minerals arriving from the outside) occur mainly in brown lesions and arrested caries, are associated with gradual rehardening and are absent in artificial demineralization.

A microscopic classification distinguishing progressive, alternating, regressive and arrested caries has been suggested. It is based on the presence or absence of areas characterizing demineralization or remineralization. Of The 500 lesions examined, show a correlation between the microscopic classification and the clinical aspect.

Penetration of radio-active calcium phosphate into the lesions has been studied in 261 cases, penetration of toluidin blue in 200 cases, using contact autoradiographs and polarized light for serial sections, and reflected light for ground surfaces.

In most lesions characterized by a mineralized superficial

zone there is no appreciable penetration of P. 32 nor stain. Carious enamel inside the lesions will take up P. 32 or stain if exposed to it directly. Lesions without the mineralized superficial zone are readily permeable to P. 32 and the stain. The mineralization of the superficial zone seems to determine the permeability of the lesion.

In a few cases of caries there is a mineralized zone at the surface and the lesion is permeable.

Artificial lesions have been produced in vitro by lactate or acetate buffer solutions. The alterations have been described. Seen in reflected light, they confirmed the interpretation of alterations due to caries. Some artificial lesions show a mineralized zone at the surface; all are permeable for P. 32 and stain.

Summary of work

Work in 1962 was confined to two phases: (1) improvement of the cariotin-injection techniques used to introduce contrast medium into the dental pulp vessels, and (2) the development of suitable methods for the examination of these vessels by projection X-ray microscopy.

Methods and Materials

Freshly extracted adult human teeth were prepared for X-ray microscopy by partially removing the crown with surgical hand saws, and severing several capillary loops within the exposed pulp to permit transcapillary aspiration of contrast

Report of the Associate Committee on Dental Research.

National Research Council.

Subject: An X-ray Microscopy Study of the Vascularisation of the Human Dental Pulp at Various Ages.

Grantees: R.L. de C.H. Saunders and H. Röckert.

Project: D.A. /05 Amount of Grant: \$ 1,050.

Introduction

In 1957 it was reported (1) that the human dental pulp vessels could be injected with contrast medium, and their vascular patterns demonstrated by contact microradiography. Subsequently it was reported (2) that the primary magnification and high resolution offered by the Cosslett-Nixon X-ray microscope made it possible to study the smallest capillaries within the human tooth.

Summary of work

Work in 1962 was confined to two phases: (1) improvement of the suction-injection techniques used to introduce contrast medium into the dental pulp vessels, and (2) the development of suitable methods for the examination of these vessels by projection X-ray microscopy.

Methods and Materials.

Freshly extracted adult human teeth were prepared for X-ray microscopy by partially removing the crown with surgical bone forceps, and severing several capillary loops within the exposed pulp to permit transcapillary aspiration of contrast

medium. This method of pulp exposure was attended by fewer injection failures than drilling, where heat tends to occlude the capillaries. The tooth was next inserted into a flexible rubber tube, which served as an injectant reservoir, and connected to a vacuum system.

Body warm ($35^{\circ} - 40^{\circ} \text{ C}$) contrast medium was then aspirated through the larger apical vessels toward the severed capillaries, using a rotary vacuum pump and negative pressure of 140-150 mm Hg. This proved to be the optimal degree of suction, although tests showed that injections were obtainable with negative pressures ranging from 40-500 mm Hg. Suction-injection was usually achieved in 10 minutes, and little or no benefit was obtained by extending the injection time up to 24 hours. To date 449 injections have been attempted with approximately 250 successful injections.

Microangiograms were obtained by injection the dental pulp vessels with contrast media of colloidal proportions, namely Micropaque and Thorotrast. The best results were obtained with the following injectants:

(a) Thorotrast Solution

Distilled water	50cc
Thorotrast	24cc
Sodium Citrate	4%
Sodium Nitrate	1%
India Ink to colour	

(b) Micropaque Solution

Micropaque	50cc
Distilled water	10cc
Sodium Citrate	4%
India Ink to colour	

Injected dental pulps were mounted on plastic film (Styrafoil 0.001 in.) for both contact and projection X-ray microscopy.

Such film is convenient for routine survey purposes but it has two disadvantages, for (1) it has an optically appreciable and calculable X-ray absorption factor, which reduces definition within the image, and (2) because the film readily takes a static charge, it accumulates dust which becomes evident at high magnifications.

When striving for high resolution it is best to dispense with the film, and support the specimen on a brass or other non-magnetic ring; this is necessary since the specimen lies within the magnetic field of the X-ray microscope polepiece.

Contact microradiography was used for survey purposes, and the X-ray microscope for magnification and examination of fine detail. The X-ray micrographs or microangiograms were taken with the Cosslett-Nixon X-ray projection microscope, using a simple metal box camera permitting varying target-plate distances (0.5-3.5 cm), and recorded on standard lantern slide plates (Kodak) or high resolution plates (Ilford), while working under atmospheric conditions. Thin copper or aluminium foil targets (3-12 μ thick), operated at accelerating voltages of 5-30 KV were used for these vascular studies. Stereographs were obtained by lateral translation of the specimen between successive exposures. Plate magnifications of x12 to x30 provided very satisfactory definition of the dental pulp capillary bed.

Discussion

The factors responsible for either partial or non-injection

of the dental pulp vessels have been studied.

Causes of failure required consideration of (1) the anaesthetic used for extraction, (2) the method of tooth preparation, (3) suction-injection pressures, (4) factors possibly promoting intravascular clotting, (5) the physical characteristics of the radiopaque injectant, and (6) pathological state of the specimen.

Teeth extracted under local anaesthetic were less likely to inject successfully than those extracted under general anaesthesia, presumably due to the presence of adrenaline causing vasoconstriction. Exposure of the pulp by fracturing the crown proved more satisfactory than drilling, since it eliminated capillary occlusion by heat. As mentioned earlier, the optimal negative pressure for suction-injection was 140-150 mm Hg.

Maintenance of the specimen and all solutions close to body temperature, proved the most important single factor conducive to successful injection. Prompt preparation of the tooth following extraction within 2 hours was important to avoid clotting, for while the specimen was immediately immersed in a body warm solution of sodium citrate (4%) and sodium nitrite (1%), this solution apparently did not permeate the whole pulp, but prevented clot and caused vasodilatation only at the root tip. Attempts to overcome clotting by preliminary perfusion with various solutions (e.g. dextran, sodium citrate, saline etc,) prior to injecting the radiopaque injectant proved unsuccessful.

Attention must be paid to the particle size, temperature, and viscosity of the radiopaque injectant. Thorotrast has a particle size of 0.1μ , and Micropaque $0.1 - 0.5\mu$, and hence

can readily enter all parts of the capillary bed. The solutions used have already been described.

Various pathological conditions may be expected to influence the facility or otherwise with which the dental pulp vessels may be injected, but at present no more can be said than that multiple pulp stones do apparently interfere with the injection of the pulpal vessels.

Conclusions

Preliminary results suggest that different vascular patterns do occur, but further studies require to be carried out in order to 1) determine the effect of ageing upon such vascular patterns, and 2) reduce the number of unsatisfactorily injected teeth. Concurrent studies on the monkey, using intravascular injections in the living animal, may be expected to show anatomical variations in the vascular pattern which may not be revealed by the suction-injection technique, and also reveal the vascular connections between the pulpal or root vessels and those in the periodontal tissues.

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APPENDIX P

Final Report to

ASSOCIATE COMMITTEE ON DENTAL RESEARCH

NATIONAL RESEARCH COUNCIL OF CANADA

on

Project DA-92

for research entitled

POLYVINYL-ALCOHOL SPONGE AND THE

HEALING OF BONY DEFECTS

by

A. H. Sinclair-Hall, M.D., F.R.C.S.

Assistant Professor

Department of Anatomy

Faculties of Medicine and Dentistry

University of Manitoba

Winnipeg, Manitoba

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INTRODUCTION

The use of polyvinyl alcohol sponge as a grafting material has had extensive experimental investigation and widespread application in a variety of surgical fields.^{1,2,3,4,5} No observations, however, have been recorded on implants of polyvinyl sponge in the types of defect here described. Investigators have reported that polyvinyl alcohol sponge (hereafter referred to as PS) is well tolerated in animal and human osseous tissues, and have found that connective tissue and bone grow into its framework.^{2,6,7,8,9} Opinions differ regarding its value as a tissue substitute.^{10,11} For example, in cardiovascular surgery, its use has been questioned because of its loss of pliability, and its calcification and fragmentation after implantation.^{12,13,14} In neurosurgery PS has been used to replace dura.¹⁵ In plastic surgery it has been used for defects of subcutaneous soft tissues of the face and breast^{5,16} and in general surgery for the repair of hernias.¹⁷ In these areas, some objection has been raised to its tendency to calcify¹⁸ and produce "wrinkling, shrinkage, and firmness" (Adler¹⁰; see also^{19,20}). However, although undesirable in soft tissue surgery, these changes in mineralization and firmness may be of value in some branches of surgery involving bone. It has been used in studies on bone regeneration,^{7,8,21,22} as an implant in cartilaginous and bony defects in joints,² and as an interposition medium in arthroplasties⁹. Oral surgeons have found it useful in the repair of various alveolar bony defects.^{23,24,25,26,27} The present investigation attempts to evaluate the long term responses of the host tissues to PS implants in a variety of bony and dentinal surgical defects produced experimentally in the jaws of dogs.

MATERIALS AND METHODS

PS (obtained as Ivalon P.V. 105, from Clay-Adams Co., New York, N.Y.) was used for implantation. Details of the properties of PS are well documented in the literature.^{4,10,18,28,29.} All the implants used consisted of uncompressed PS cut from one large block prepared as described in the manufacturer's directions. These pieces were autoclaved for 5 minutes at 135°C. under 27 pounds pressure and then stored in 1:1000 zephiran chloride solution. No implant was stored longer than one week. Each implant was carefully washed in sterile isotonic saline before use.

The 17 healthy young adult mongrel dogs used weighed on the average 6.6 kilograms (range 3.0 - 9.0), and had healthy gums and teeth as determined by visual inspection. The dogs were housed individually and fed a balanced mixed diet of fox food and tinned meat. Each animal was anaesthetized by Nembutal sodium given intravenously (Pentobarbital sodium, Abbott Laboratories, North Chicago, Illinois). A single intramuscular injection containing 300,000 i.u. of procaine penicillin G, 100,000 i.u. of Sodium penicillin G and 0.5 gms. of streptomycin sulphate either preceded or followed anesthetization.

The general experimental procedure consisted of producing defects or wounds of three types (see below) in the upper or lower jaw of each dog on both sides. The defects on the left side were filled with PS, those on the right side were left as unfilled controls.

For sites of implant and control defects see Fig. 1a. In this figure, the arrows numbered 1 to 4 on the dog's right, indicate the sites

of the control bony defects and the arrows numbered 5 to 8 on the dog's left indicate those of the implants. The 3 different types of experimental defect as indicated by the numbered arrows in Fig. 1a are:

Type I - (see arrows 2 and 6) maxillary second incisor socket defects in cancellous bone. These simulate cystic, exodontic or other surgical loss of bone.

Type II - (see arrows 1 and 5 or 4 and 8) maxillary or mandibular circular defects situated in compact bone of the canine eminences. These simulate periapical alveolar bony lesions.

Type III - (see arrows 1 and 5 or 4 and 8) similar to Type II but extended through compact bone, periodontal membrane and cementum into root dentin. These simulate more extensive resorption or surgical loss.

These defects will hereafter be called Type I (socket cancellous), Type II (compact), Type III (compact and dentinal). Type II and Type III defects were all circular in shape except in one animal in which special Type III defects rectangular in shape were made in interdental compact bone and extended into dentin (see arrows 3 and 7). These defects will hereafter be called Type III (bx). These simulate interdental bone loss extending into dentin.

The implants were of two kinds: (1) Inlays embedded subgingivally in the Type I defects and (2) Combined inlays and onlays embedded submucoperiosteally in the Type II and Type III defects.

Under aseptic technique and using an oral approach the various PS implants were inserted, either through extraction wounds in the gum or through a transverse mucoperiosteal incision over the canine eminences,

and were embedded in the defects without fixation. The wounds over the implant and control defects were closed with no. 0000 silk interrupted sutures on a curved atraumatic needle.

The operative procedure for each type of defect was as follows, the shape of each of the three types of implant and bony defect being shown diagrammatically in Fig. 2 (implants black).

Type I (socket cancellous) - Defects approximately 6 x 4 x 2 mm., were produced by extracting both left and right second incisor teeth. PS was cut to fit the left socket defect snugly and was inlaid without being compressed. The control socket on the right remained untreated and the gingiva was sutured as on the left (Fig. 1b).

Type II (compact) - After elevating and reflecting a mucoperiosteal flap from the underlying bone, circular defects, approximately 5 mm. in diameter by 2 mm. deep, were cut slowly in compact bone with dental burs (Fig. 1c). To facilitate later identification, the center of each hole was placed a measured distance from the canine incisal surface. On the dog's left, PS was cut to fit into the defect, overlap its margins and protrude beyond the surface of the cortical bone as a combined inlay and onlay graft (see Fig. 1d arrow). The cut PS implant was mushroom shaped, the stem of the mushroom fitting into the defect, the umbrella overlapping the defect margins by approximately 2 mm. Its summit protruded 2 mm. beyond the original preoperative contour of the bone (see Fig. 2 showing shape of Type II implant and defect). The implant was purposely cut larger than the defect in order to observe any outgrowth of tissue into the PS that might reshape the bone (Fig. 1d arrow). After PS was implanted subperiosteally, the wound was closed over both implant and control defects (Fig. 1e).

Type III (compact and dentinal) - Circular defects were cut as in Type II but deepened through compact periapical bone, periodontal membrane and cementum into dentin. These holes measured approximately 5 mm. in diameter and were 4 mm. deep. The mushroom shaped PS implant was cut as in Type II but with a longer stem (Fig. 2).

Type III (bx) - After raising the mandibular mucoperiosteal flap further, but leaving the free gingival margin undisturbed, special rectangular vertical defects (Fig. 1a, 3 and 7) approximately 4 mm. long, 2 mm. wide and 3 mm. deep, were cut in the compact interdental bone of the alveolar crests between the second and third incisors on both sides; these defects were extended into the dentin of the third incisor, also on both sides. A smaller mushroom shaped PS implant was cut and inserted as in Type III (Fig. 2 (bx)). Both rectangular and circular defects were cut in the same animal.

After operation the dogs were given a fluid diet for 2 days and then allowed soft food. Their wounds were examined daily for inflammatory reaction during the first postoperative week, and weekly thereafter until healed. Changes in shape, contour or consistency of the implant or control regions were followed clinically. At intervals of from 3 to 7 1/2 weeks, dogs were killed by an overdose of Nembutal Sodium (see Fig. 2 for individual times).

The defects were cut out immediately after death and fixed in formalin, decalcified and embedded in paraffin. Vertical labio-lingual sections were cut at 7 μ and stained with hematoxylin and eosin. Some sections were stained with Mallory's phosphotungstic acid. When PS was

not found in routine sections, serial sections of the defect were examined. Representative microscopical sections were photographed.

Röntgenograms on regular dental films were made of the PS and control defects after excision but before decalcification.

A graft was considered successful when retained firmly in its bed, and if histological examination revealed that connective tissue or connective tissue and new bone penetrated the sponge.

RESULTS

The healing of the three different types of defect was studied clinically, roentgenographically and microscopically.

1. Clinical Observations.

One dog died postoperatively of an anesthetic overdose. The 16 surviving animals remained healthy until killed at the times shown in Fig. 2. The implanted PS was found in 13; in these the gingival and mucosal wounds healed rapidly and were remarkably free from inflammatory reaction. Weekly examinations of the healed wounds revealed no appreciable difference in shape, contour or consistency between the implant and control regions during the entire postoperative period.

A representative photograph taken 36 weeks after maxillary and mandibular implantations of the PS shows this similarity (Fig. 1f).

The implanted PS was not found in any type of defect in 3 animals, those killed at 5, 23, and 41 weeks respectively. In these, small areas of the gingival and mucosal wounds became ulcerated, taking 2 weeks to heal. The PS was not seen in any of these ulcerated areas and could have been extruded unobserved.

2. Roentgenographic Observations.

All Type I (socket cancellous) defects up to and including those in dogs killed at 31 weeks post-operatively revealed apparent radiolucency and delay in development of the normal trabecular pattern on the implant side (L), as compared to the controls (R)(Fig. 3, a & b).

There was no evident difference at 32 and 36 weeks.

At 71 weeks (Fig. 3c) and 74 weeks there appeared to be a less distinct trabecular pattern and slightly increased density on the implant side (L).

In spite of the use of various techniques, roentgenograms of the Type II and III cortical bony defects were obscured by the greater density of the root shadows.³⁰ This made impossible a comparison similar to that for Type I. However it was possible to observe that the 74 week Type III (compact and dentinal) mandibular defect on the implant side showed a radiopaque area external to the cortical plate. That this may have been due to calcification in the implant is suggested by Schwartz's¹⁸ finding that PS subcutaneous implants in dogs contained calcium deposits and were radiopaque in contrast to radiolucent pure polyvinyl-formal material.

3. Microscopic Observations.

(a) Comparison of the effect of time on implant and control defects.

Type I (socket cancellous) - See Fig. 2 for post-operative duration of successful implants.

The 3 and 4 week implants showed bridging of the alveolar crests with new bone. The sponge was not penetrated by bone, but was penetrated by abundant immature and maturing connective tissue containing areas of

aseptic inflammatory reaction. Multinucleated giant cells were present at the sponge-tissue interface (Fig. 4).

At 6, 9 and 29 weeks the reaction was fibroblastic. At 31 weeks bony remodelling in the implant had become active and immature bone had completely penetrated the defect. This stage of repair, as will be seen later, was present in the 3 week control.

At 31 weeks irregularly shaped masses of foamy appearing sponge outlined by a thick dark basophilic border were observed, surrounded by and interlocked with immature bone and collagenous tissue (Fig. 5). Profuse collagenous and bony penetration was present, the new bone formation in the PS having the typical appearance of endochondral bone formation as seen in long bones.

At 36 weeks there was less osteoblastic and osteoclastic remodelling. The degree of bony repair was equal to the 6 week control. The interspaces of the PS were penetrated by mature connective tissue and bony trabeculae which had reached the center of the PS and appeared to anchor it to the host bone (Fig. 6). The periphery of the socket was in some areas occupied by fatty marrow. Again the appearance typical of endochondral bone formation was seen. The bony trabeculae were smaller and there appeared to be less bone in the implant defect than in the corresponding control (Fig. 7).

At 71 weeks a stable bone condition was present and mature bony trabeculae were interlocked with PS. Slight reduction in size of the PS had occurred but no degenerative changes were observed in the tissues or the implant. There was a striking diminution in collagenous tissue and

a no less striking preponderance of normal mature fatty marrow in the interspaces (Fig. 8).

At 7 1/2 weeks (Fig. 9) bony trabeculae and profuse collagenous connective tissue were present in the interspaces. There was no fibrous encapsulation of the PS within this or any other socket. There now appeared to be more bone in the implant defect than in the control (Fig. 10). Interlocking and interdigitation of the 7 1/2 week implant with the bone was seen under higher magnification (Fig. 11a). The interdigitating bone gave the appearance of compressing the PS. The connective tissue and bone remained limited to the interspaces and did not penetrate the foamy appearing fine closed pores of the sponge mass. The sponge and tissues seemed to be in harmonious contact, and there was no clear evidence of deterioration in either implant or host tissue at 18 months.

Multinucleated giant cells were situated at the sponge tissue interface in all implants. Areas of primitive connective tissue similar to that seen in Fig. 11 a & b were present in all implants and in many of the controls. The darkly stained basophilic margin of the sponge was thicker in the older (71-7 1/2 week) implants, and the sponge seemed to occupy a slightly smaller area of the socket than in the younger (3-36 week) implants.

At 5, 7, 10, 20, 23, and 41 weeks the implants were not found (Fig. 2). Their sockets did not differ from the controls of equal post-operative duration, except that one unsuccessful graft socket at 5 weeks showed more aseptic inflammatory reaction and less bridging of the crest than did its corresponding control.

The 3 week control showed a degree of repair similar to that present in the 31 week implant. Active remodelling was in progress and new bone bridged the alveolar crest and completely penetrated the defect. At 7 weeks a mature stable trabecular pattern within fatty marrow had been established; this persisted from then on. The histological picture for all the remaining controls was similar to this 7 week picture.

Observations of special interest in the Type I implants were:

- (1) Bone formation was delayed, prolonged and appeared to be increased in amount at 18 months (Figs. 9 and 10).
- (2) New bone, developing from connective tissue on the sponge framework, simulated the appearance of new bone forming on the cartilage framework in endochondral bone growth (Fig. 5).
- (3) The repair of the sockets in which implants were not found differed but little from that in the corresponding controls.

Type II (compact) - For post-operative duration of successful implants see Fig. 2.

The implants of this type healed more slowly than Type I. Those found up to and including the 9 week specimen (Fig. 2), were lined by immature bone, and both the inlaid stem and onlaid umbrella of the mushroom shaped implants were penetrated by maturing connective tissue. The 4 week specimen showed muscle fibers penetrating the sponge (Fig. 12). In sections from this specimen stained with hematoxylin-eosin and with Mallory's phosphotungstic acid haematoxylin, high magnification showed muscle striations quite clearly. Muscle penetration of the sponge occurred to a lesser extent in several other Type II (and Type III) specimens. In the 9 week specimen young fat cells were present in the center of the sponge (Fig. 13); similar cells were observed in other Type II implants.

The only implant of this type older than 9 weeks was the 71 week specimen (Fig. 14). This specimen is of particular interest because while mature bone penetrates into the stem of the mushroom shaped implant only as far as the original preoperative contour of the bone, profuse collagenous tissue extends into the umbrella of the sponge beyond the original bony contour. This fibrous recontouring was present 18 months after implantation.

Fig. 2 shows the experiments that were not performed. It also indicates the cases in which the implants were not found. These defects did not differ from the controls, of corresponding ages, described below. In the 29, 31 and 36 week specimens there was no soft tissue opposite the healed bony defects. These implants may not have been found because of technical errors.

The healing of these controls was much slower than that of the controls of Type I. At 9 weeks the defects were lined by immature bone and filled with mature connective tissue and at 29 weeks, and older, they were filled with mature bone.

Observations of special interest in the Type II implants were:

- (1) Muscle fibers penetrating the sponge (Fig. 12).
 - (2) Young fat cells in the center of the sponge (Fig. 13).
 - (3) Fibrous recontouring (Fig. 14).
- Type III (compact and dentinal).

Observations of the Type III defects were made at 3, 4, 10, 20 and 74 weeks (Fig. 2).

The bony walls of the 3 and 4 week implant defects were lined with immature bone. The original excavations in their dentinal floors were lined by cellular cementum. Both the inlaid stems and onlaid umbrellas

of the mushroom shaped implants were penetrated by immature connective tissue. This connective tissue was continuous with the periodontal membrane, and lined all the boundaries of the defects including the cementum of their floors.

The 10 week specimen (Fig. 15) showed a similar picture and still no bony bridging of the defect was present. But the connective tissue penetrating the PS was more mature. The parallel collagenous fibers lining the bony defect were continuous with the reattaching periodontal membrane in the area of the cementum lined dentinal defect outlined by the square in figure 15. Reattaching periodontal membrane, proliferating connective tissue, cementoblasts and PS were seen in intimate contact with one another in a higher magnification of the outlined area (Fig. 16).

The 20 week specimens, both circular Type III and rectangular Type III (bx) showed active remodelling and penetration of all parts of the implant by immature trabeculae. The new bone bridging both these types of defect was seen to bulge out into the subperiosteal umbrella of the PS beyond the original preoperative contour line of the cortical bone, producing bony recontouring, as indicated by the arrow in Fig. 17. This temporary bony recontouring, present in the 20 week circular specimen, was not present in the 7½ week circular specimen. In both Type III and Type III (bx) 20 week specimens mature connective tissue also penetrated the PS, and mixed cellular and acellular cementum lined the dentinal defects.

In the 7½ week specimen mature bone, bridging the defect, had re-established the original cortical bony contour. The onlay part of the implant was penetrated and surrounded by mature connective tissue extending

out beyond the bone into the soft tissues; thus was produced a fibrous recontouring of the bone.

In the controls of this group, healing was much slower than in corresponding Type I controls. The walls of the 3 and 4 week control defects were lined by immature bone, and their floors by new cellular cementum. Immature connective tissue filled these defects, and was continuous with the tissues lining their boundaries. At 10 weeks the connective tissue was mature, by 20 weeks the defect was two-thirds filled by bone, and at 74 weeks it was completely filled. The cementum, partly filling the excavations into the dentin, was of mixed cellular and acellular type in the 10, 20 and 74 week specimens.

All Type III implants were successful. Observations of particular interest in the Type III implants were: (1) The presence of new bone and proliferating connective tissue in implants in contact with periodontal membrane, the latter having reattached to newly formed cementum lining the excavations into the dentin (Fig. 16). (2) Fibrous recontouring. (3) The temporary bony recontouring present in the 20 week specimen (Fig. 17).

(b) Comparison between different types of defect of the same age.

Although the study was designed to make comparisons of the type made in section (a) above, it is possible from the data to make a few comparisons between defects of different types of the same post-operative duration. These are given below.

(1) All Type I, 3 week defects healed more rapidly than corresponding Type II or Type III defects of the same age. (2) The Type I, 3 week implant defect healed more rapidly than the corresponding Type III. (3) The Type I,

4 week implant defect healed more rapidly than the corresponding Type II or III. (4) The Type I, 6 and 9 week implants healed more rapidly than the corresponding Type II. (5) The Type III, 20 week implant healed more rapidly than its corresponding Type I implant, being penetrated by bone at 20 weeks whereas the Type I was not penetrated at 29 weeks. (6) The Type I, 71 week implant healed more rapidly than the corresponding Type II. (7) The Type I and Type III, 74 week implants both showed complete healing.

Complete bony penetration of the sponge occurred most rapidly in compact bony and dentinal defects. Penetration next in rapidity occurred in cancellous defects, the least rapid in compact defects.

The type of tissue penetrating the implant varied with the type of defect and with the postoperative duration of the implant. 60.1% of implants were successful on the basis of the criteria already specified.

DISCUSSION

Fresh autogenous bone grafts are at present favoured in the healing of bony defects, but they have the disadvantage of requiring an additional operation. In an attempt to obtain an easily procurable synthetic substitute, several studies have been carried out on PS. Almost all these studies have been made over relatively short periods of about 6 months^{2,7,8,22,24,25,26,27}; the longer term effects and the tissue changes that occur in control defects are not so well known.

Consequently, the present investigation was designed to evaluate the long term responses of the host tissue to PS implants in several new types of bony and dentinal defect produced experimentally in the jaws of dogs. These types of defect, to the author's knowledge, have not previously been implanted with PS. The observations made in the present experimental

study suggest certain clinical uses of this material.

The finding in Type I (socket cancellous) implants that an apparently increased amount of bone and calcified sponge is present at 18 months, suggests that PS may be of value for healing and preventing resorption of alveolar ridges following cystic, exodontic or other surgical loss of bone.

The finding in Type II (compact) and Type III (compact and dentinal) implants that abundant fibrous tissue reshapes the outline of the bone for at least as long as 18 months, suggests the use of the sponge (a) for restoration of original contour where loss of bone has occurred, and (b) for creating fibrous pads where required in surgery, e.g., rebuilding and cushioning resorbed alveolar ridges for denture retention, in edentulous patients.

The finding in Type III (compact and dentinal) implants of proliferating fibrous tissue continuous with periodontal membrane in contact with reforming cementum lining excavations into dentin, suggests the use of the sponge in more extensive surgical wounds involving tooth root tissue, and possibly for assisting periodontal reattachment.

The finding of muscle fibers, that depressed the lower lip and angle of the dog's mouth, in Type II and Type III implants suggests the possibility of a movable soft tissue facial prosthesis.

Certain observations require further comment:

1. Those dealing with the repair of bone. The early delay in bone formation during the first nine months in the Type I implants may be the result of mechanical interference with the blood clot by the PS. This

prolongation of repair may also depend upon the necessity for additional remodelling of trabeculae in accordance not only with the demands of stress but to conform to the shape of the "artificial" sponge model, in and on which the new bone growth takes place. This combination of influences in healing may account for the temporary bony recontouring observed. Besides mechanical factors the aseptic inflammatory reaction possibly delayed trabecular osteogenesis.

The presence of the sponge in socket defects following tooth extraction may be a substitute for the stimulus of functional stress in maintaining prolonged repair and preventing later resorption of alveolar bone.

The superficial extent of penetration of the PS by bone in Type II (compact) defects as compared to the complete penetration in Type I (socket cancellous) defects is in accordance with the normal remodelling of bone to its original contour.

The Type III and Type I defects heal much more quickly than Type II, possibly because of their better blood supply; perhaps also because of their extension into endosteal and more primitive marrow and tooth attachment tissues that possess greater osteogenic potential. In contrast, the walls of the Type II defects are formed of more differentiated cells of compact bone and periosteum on the canine eminences. A study of healing in isolated cortical defects by Bassett et al.³¹ showed that the endosteum contributes a large part of the cells responsible for osteogenesis in the dog.

Dukes²⁰ observation of ossification in fibrous tissue that had invaded PS, 24 months after implantation subcutaneously in the rat, is of

interest as the sponge may have been acting as an artificial substitute for a cartilaginous or bony model, thus making possible heterotopic bone formation from connective tissue.

2. Those dealing with fibrous tissue formation in and on the sponge.

Whether or not these fibrous pads will retain their shape and hold up under the functional strains imposed at various bony implantation sites requires further investigation. Fibrous tissue is the packing material of the body and is functionally adaptable to changes in pressure and strain. Following satisfactory experiments in dogs, Cobey and others,⁹ interposed PS between the joint surfaces of arthritic knees in two patients and reported them walking and the sponge holding up well for observed periods of 9 and 7 months.

The presence of abundant fibrous tissue in PS may be attributed to irritation caused either by the sponge itself or by the fluid absorbed into the sponge. Fibroplasia in association with lymphoedema, following lymphatic obstruction, is well known.

3. Those dealing with various cell types. The presence of cells in the center of the implants looking exactly like young fat cells, and not like degenerated cells, could be explained by differentiation of primitive cells within the sponge. Bassett and Herrman³² showed in tissue culture studies that cells with osteogenic capacity respond to certain environmental factors by producing bone, cartilage or fibrous tissue. In this study it would appear that cells of this type may also have produced fatty tissue. If differentiation of fat cells is possible within the sponge, differentiation of fibroblasts into osteoblasts may likewise be possible.

Profuse formation of fibrous tissue occurred in and about the adjustable framework of the implants, and produced a planned fibrous reshaping beyond the original bony contour; this was observed up to and including 18 months. There was little evidence of deterioration either in implants or in host tissues.

Among observations of special interest were (1) muscle fibers penetrating implants, and (2) implants, containing proliferating fibrous tissue and new bone, in contact with periodontal membrane reattaching to newly formed cementum lining defects in dentin.

These observations suggest interesting lines of further investigation. Various possible clinical applications have been suggested.

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Fig. 1 - Dog's jaws.

- a. Numbered arrows on the dog's left indicate the implant defects and those on the dog's right the controls.
- b. Gingivae sutured over Type I (socket cancellous) maxillary implant and control defects.
- c. Circular Type II (compact) defects prepared in mandibular bone.
- d. Sponge implant (arrow) in position on dog's left, just before closure of mucoperiosteal flap.
- e. Mucoperiosteal flap closed.
- f. Representative photograph showing similarity in contour of maxillary implant and control alveolar areas 36 weeks post-operatively. The mandibular mucosal scar is also seen.

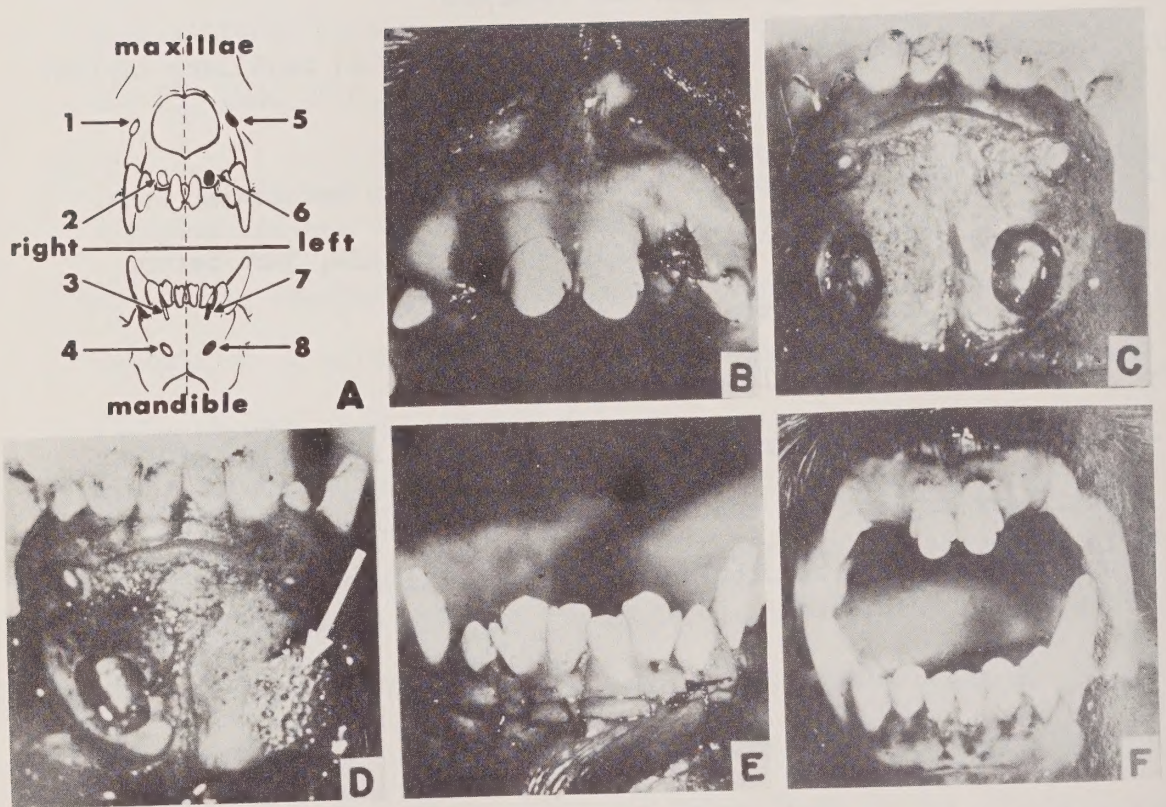


FIG.1

NATIONAL RESEARCH COUNCIL

ASSOCIATE COMMITTEE ON DENTAL RESEARCH

Annual progress report for 1962-63

Grant #DA-94

Grantee: Jules Tuba, Faculty of Dentistry, University of Alberta

Topic I

A year ago we reported on studies at the University of Alberta on the nature of the cariostatic action of dietary phosphates. The studies were planned to determine whether cariostatic effects are local, systemic or both. Weanling hamsters were fed the cariogenic diet of Nizel and Harris (1). Other groups of growing hamsters were maintained on this diet plus supplements of phosphorus supplied as Na_2HPO_4 , which brought the phosphorus level of the cariogenic diet up from 0.4% to 2 and 3 times this value. Among the information obtained was the effect of the cariogenic diets and of the supplemented diets upon salivary phosphorus values. This past summer we attempted to obtain more convincing evidence on this matter and considerably modified the procedures.

Three diets were used:

- (a) A "normal" non-cariogenic diet:
Powdered skim milk, oatmeal, commercial Purina checkers
Phosphorus assay = 1.3%
- (b) The cariogenic diet of Nizel and Harris(1):
Phosphorus assay = 0.4%
- (c) Diet (b) + 0.8% phosphorus as Na_2HPO_4 :
Total phosphorus = 1.2%

Adult hamsters were maintained on these diets for several weeks and saliva was collected periodically and assayed for phosphorus. Before saliva was collected, the animals were deprived of food for varying periods of time (one to 18 hours), to determine the best postprandial hour for obtaining saliva. Six animals were used for each diet.

Various methods of saliva collection were tried and we decided to use cotton swabs. An absorbent cotton swab on one end of a glass rod was used. The swab and rod were placed in a 30 ml. Kjeldahl flask and these were weighed. The animal under examination was lightly anesthetized in a plastic-covered 1,000 beaker into which ether fumes were forced by a current of air through a rubber tube. (Ether was injected by syringe into the air tube.) The swab was used to mop up saliva in the mouth and in the pouches. Pouches were swabbed last to permit rapid removal of the rod if the animals recovered from the light anesthesia (which lasted about 30 seconds). The wet swab on the rod was placed

(1) International Association for Dental Research, page 11, 1961.

back in the original Kjeldahl flask and reweighed to give the weight of saliva. Samples of saliva were 60 - 100 mgm.

Last year we treated saliva with trichloroacetic acid and estimated inorganic phosphorus in the supernatant after centrifugation. Afonsky (2) reports that great discrepancies exist in the literature due to varying techniques, so we felt it would be better to determine total phosphorus (organic and inorganic) present in a sample. So the sides of the flask were rinsed with a few ml. H_2O and one ml. concentrated H_2SO_4 was added. The flask was heated on a digestion rack until charring occurred. Sufficient 30% H_2O_2 was added to clear the digest which was heated until H_2SO_4 fumes appeared. The flask was cooled and its contents were transferred to a 25 ml. volumetric flask.

Phosphorus was determined colorimetrically by the method of Fiske and Subbarow. Readings were made with a DU Spectrophotometer at 680 mu with 0.03 mm. slit. 660

In addition to reagent blanks we used dry swabs which were ashed and phosphorus estimated, as controls. Further, a swab was dipped into a solution of KH_2PO_4 containing 0.08 mgm/ml. This was weighed, ashed, and recovery of phosphorus was estimated. Recovery was slightly below 100%, but was allowed for in standard curve.

Results: Are reported in the following table.

TABLE I - THE EFFECT OF DIET ON HAMSTER SALIVARY PHOSPHORUS

	<u>Fasting Period (hrs.)</u>	<u>Number of Samples</u>	<u>Average P mgm/100 gm</u>	<u>Range P mgm/100 gm</u>
<u>Diet A</u>	1	30	7.4	6.4 - 11.8
(1.3% P)	6	12	8.95	4.4 - 16.1
	18	16	8.6	7.9 - 9.4
<u>Diet B</u>	1	30	5.7	6.4 - 7.4
(0.4% P)	6	12	6.6	4.4 - 8.1
	18	16	6.1	4.3 - 7.1
<u>Diet C</u>	1	30	7.4	5.1 - 10.0
(1.2% P)	6	12	11.4	5.5 - 17.5
	18	16	10.2	8.5 - 12.5

(2) Afonsky "Saliva and Its Relation to Oral Health", University of Alabama Press, 1961.

Discussion:

The results in the table indicate that there is a narrower range of results if the animals are fasted 18 hours rather than for 1 hour or 6 hours. The diet containing the highest concentration of phosphorus appeared to affect most salivary levels of phosphorus.

It was our experience that the hamsters fasted for shorter periods of time were more likely to retain food in their cheek pouches. This tended to give an abnormally high salivary phosphorus level and accounts for the wider range of results after the shorter fasting periods.

Our experiments with weanling hamsters during 1962-63 made use of the three diets above. We ran into the problem of caries resistance (not encountered during the experiments of the preceding year). Caries scoring was impossible to carry out with confidence, so we attempted to salvage the experimental material by examining the enamel shells of molars for calcium phosphorus ratios in the groups on three diets. This involved considerable work, but several checks on ratios proved that the calcium phosphorus ratio was about 2:1 in all three dietary groups.

Conclusions:

1. Caries resistance appeared in our hamster colony and this made it impossible to continue our investigations concerning the influence of dietary phosphorus and salivary phosphorus levels upon the incidence of dental caries. Another complication was food retention by hamsters in their cheek pouches.
2. It was necessary to change our approach to this whole problem and it was decided to use rats of the Sprague-Dawley and Rochester caries-susceptible strains. This change in tactics appears to be a way to a promising start and is discussed in Topic II.

Q - 4

NATIONAL RESEARCH COUNCIL

ASSOCIATE COMMITTEE ON DENTAL RESEARCH

Annual Progress Report for 1962-63

Grant #DA-94

Grantee: Jules Tuba, Faculty of Dentistry, University of Alberta

Topic II Dietary Phosphate and Levels of Phosphorus in Rat Saliva.

Introduction:

Our experiments on the cariostatic action of dibasic sodium phosphate (Na_2HPO_4) in hamsters were concluded this summer. It was felt that since 1948, the protective action of dietary phosphate has been clearly shown to produce caries resistant tooth composition. However, problems still remain regarding the relationship between dietary phosphate, caries resistance, and composition of body fluids, notably saliva.

Experiments to provide information on this last factor, saliva, were begun with hamsters, but were abandoned because of certain problems described in the section dealing with the preliminary studies with these animals.

Experiment:

Animals used are adult male rats of the Sprague-Dawley Strain. These are housed in individual stainless steel metabolism cages, which are constructed in a manner which permits collection and separation of feces and urine. The animals ordinarily are maintained on a diet consisting of powdered Purina Chow Checkers and water ad libitum.

Our first problem was collection of unstimulated saliva in micro amounts which could be measured accurately. A survey of the literature disclosed a paucity of techniques, although some use has been made of the Lashley cup (Lashley, K. S., J. Exptl. Psych. 1:461-493, 1916) or modifications. This method was not suitable for our rat studies. Several absorbent materials were tested and finally we found dental cotton pellets to be most satisfactory. The mouth of the animal is held open with a device made in our laboratory for this purpose. Essentially, this means that a wire loop is hooked over the upper incisors and the mouth is opened sufficiently to expose the tongue. One cotton pellet is placed with fine, bent tweezers under the tongue and left for a minute or two until wet. A second pellet is then used in a similar way with the same animal; the top of the tongue and cheeks are swabbed as well.

In this way it is possible to obtain samples of saliva about 50 mgm as an average. The weight of saliva is determined by the difference in weight of the wet and dry cotton pellets. The two pellets used for each animal are dropped at once into 15 x 125 mm. Pyrex tubes to avoid loss by evaporation. To each tube is added one ml. 5M. H_2SO_4 . In order to have sufficient phosphorus present to give reliable optical density readings on the spectrophotometer, we add 1 ml. of phosphorus standard (0.05 mg P/ml.). The tube and contents plus two glass beads are heated on a boiling water bath until the cotton pellet disintegrates; this facilitates the subsequent digestion on a micro Kjeldahl digester at a moderate temperature. The charred contents of the tube are treated with 5 drops of 30% hydrogen peroxide and heated until sulphuric fumes appear and the digest is clear. Control tubes are run with pellets only to test for phosphorus content, and tubes with 1 ml. standard only.

To the clear liquid one adds 3 - 4 ml. water, rinsing the sides of the tube at the same time. Addition of 2.5 ml. of 2.5% ammonium molybdate with rapid mixing is followed by 1 ml. of aminonaphthosulfonic acid reagent. After mixing, the contents of the calibrated tube are diluted with water to 10 ml. This volume was the minimum we could use without some reagent (probably ANSA) crystallizing out. The blue colour is read in the Beckman DU Spectrophotometer within 10 minutes at 660 m μ against a reagent blank.

Note:

The above technique permits use of one tube only and avoids loss of phosphorus by transfers from one flask or tube to another.

Results:

Thirty-three samples of saliva from adult non-fasted male rats gave a range of phosphorus = 7.8 - 16 mgm% with an average of 10.9 mg%. S.D. = 2.66 and S.E. = 0.56.

Force feeding:

Animals are deprived of food, but allowed water overnight. In the morning they are force-fed one ml. $\text{Na}_2\text{HPO}_4 \cdot 7\text{H}_2\text{O}$ containing 0.024 mgm P/ml. Force feeding was performed with the aid of a syringe and #17 needle. The 5 cm. needle was blunted at the sharp end with a bead of solder and bent slightly at this end to facilitate passage of the needle down the esophagus to the stomach. We have now acquired considerable dexterity in force-feeding without regurgitation of the phosphate solution. A drop of dye is added to the phosphate solution to indicate when regurgitation occurs.

One hour after force feeding a sample of saliva is obtained from the animal in the usual way.

Results of force feeding:

A few results have been made available for this report. Thirteen samples of saliva had a range of phosphorus = 19.4 - 49.9 mgm%. Average = 33.9 mgm%. S.D. = 11.1 and S.E. = 3.01.

These preliminary observations show that force feeding Na_2HPO_4 to fasted animals produced a marked elevation in salivary phosphorus levels.

Muhler (1) reports that the phosphorus content of resting mixed human saliva is 13.6 - 15.5 mgm/100 ml. Afonsky (2) states that values of human salivary phosphorus vary greatly in the literature due to the great variation in techniques. Lura (3) gives a range for phosphorus in human unstimulated saliva of 12 - 18 mgm%.

In the case of our experiments with rats the salivary values compare reasonably well with the ranges reported for humans.

Experiments now being carried out and those being planned:

1. More values for unstimulated salivary phosphorus will be obtained from animals on a normal laboratory diet. *and effect of number of food-bidings and optimum time of sampling will be determined*
2. Calcium levels in these saliva samples will also be estimated. Cotton pellets wet with saliva will be wet-ashed as above and calcium is to be determined in the clear digest as the phosphate.
3. Urinary phosphorus and calcium levels will be determined. A beginning has been made on this.

- (1) Textbook of Biochemistry for Students of Dentistry, C.V. Mosby Co., 1959.
- (2) Saliva and Its Relation to Oral Health, University of Alabama Press, 1961.
- (3) Odontologiske Undersogelser over Spytets Enzymer, Munksgaard, Copenhagen,

DISPERSION STRENGTHENED AMALGAMS

by
D. B. K. Innes*
and
W. V. Youdelis**

University of Alberta
Edmonton

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Sponsored by the National Research Council of Canada Under
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*Graduate Student, Department of Mining and Metallurgy.

**Assistant Professor of Metallurgy, Department of Mining
and Metallurgy.

DISPERSION STRENGTHENED AMALGAMS

SUMMARY

An attempt has been made to improve the mechanical properties of dental amalgams by the application of dispersion strengthening principles.

The dispersion phase used in this study is a silver-copper eutectic alloy (71.9% Ag - 28.1% Cu).^{*} A dispersion strengthened amalgam is prepared by adding the dispersion phase powder to a standard alloy powder (75% Ag - 25% Sn atomic percent) in various weight ratios, and then amalgamating the powder mixture with a specific alloy to mercury ratio. An amalgam of the standard alloy without dispersion powder is used as a reference standard. A commercial amalgam, with and without dispersion powder, has also been tested. Evaluations of the dispersion strengthened amalgams have been made according to the American Dental Association's Specification No. 1 for the dental amalgam alloy.

Results to date show that flow of the standard amalgam can be reduced by more than 600% and that the compressive strength is increased by more than 30% by additions of the dispersion phase powder of appropriate particle size and amount. Flow of the commercial amalgam was decreased by approximately 100% and compressive strength increased by approximately 15% by adding the dispersion powder. Biological tests indicate that the dispersion strengthened amalgams have no adverse toxic effects. Clinical testing of the dispersion strengthened amalgams is to begin shortly.

For future studies it is proposed that a fundamental study of dispersion hardened amalgams be continued to:

^{*} All concentrations in weight percent unless otherwise specified.

- (1) Determine the nature of the bonding between the dispersion particle and the amalgam matrix using X-ray diffraction techniques.
- (2) Determine the effects of smaller dispersion particle sizes.
- (3) Investigate behavior of alloy systems other than silver-copper as the dispersion phase.

INTRODUCTION

(1)
Dispersion strengthening of metals is a fairly recent metallurgical development and has been used mostly to improve the high temperature strength of some alloys.

Deformation of metals involves slip with which is associated the movement of dislocations through the crystal lattice. Dislocations can be described generally as lines of imperfection in a metal crystal. They can form as a result of stacking faults occurring during solidification of the metal or during cold work. If obstacles are placed in the paths of these dislocations, with which there may be associated an elastic stress field, more energy is required to move the dislocation past the obstacle, and so higher stress levels are achieved for a given amount of strain. This is the basis of dispersion strengthening.

MATERIALS

The dispersion phase used in this study is the silver-copper eutectic alloy (71.9% Ag - 28.1% Cu); its phase diagram (2) is shown in Fig. 1. A basic requirement for a dispersion phase is that its hardness and strength exceed that of the matrix material. The hardness and strength (3) of the silver-copper eutectic alloy is derived from the very fine lamellar structure of the α and β phases obtained on quench casting the alloy (see Fig. 2). The toughness of this alloy makes it difficult to powder and this was possible only by a laborious filing technique. Other reasons for choosing this alloy as the dispersion phase are its low susceptibility to corrosion and low toxicity, since it is a noble alloy, and its tendency to amalgamate, forming a diffusion bond with the matrix. Only the surface of the particle amalgamates leaving the major volume of the particle for strengthening the matrix.

An amalgam of 75% silver - 25% tin (atomic %) alloy (to be referred to as the standard alloy) with a specific alloy to mercury ratio was chosen as a reference standard. The microstructure of this standard amalgam (see Fig. 3) does not differ much from the amalgamated commercial alloys. The microstructure of the dispersion strengthened amalgams (Fig. 4) is a mixture of the amalgamated phases of the standard silver-tin alloy and the silver-copper dispersion alloy. These amalgams are prepared by adding an amount of dispersion powder equal to some percentage by weight of standard alloy powder present (referred to in terms of weight ratios). The total powder weight is then amalgamated using a

* All concentrations in weight percent unless otherwise specified.

specific alloy powder to mercury ratio. All the amalgams reported here were prepared in a Wig-L-Bug amalgamator using a metal capsule and a ball bearing pestle. In all the tests reported using the standard alloy matrix, the alloy particle size range is $(-103 +74)$ microns or $(-150 +200)$ mesh. The condensation pressure used in all tests was 10,000 p.s.i., applied for one minute using a Hounsfield Tensometer.

TESTING PROCEDURES

Flow of dispersion strengthened amalgams was determined ~~in~~ by the procedure set down in the American Dental Association's Specification No. 1, with the exception that the diameter of the specimen was 0.1563 inches rather than four millimeters (0.1575 inches). Figure 5 shows the flow test machine. The load is applied with a fulcrum mounted beam; the specimen sits on a stage on one end of the beam and the flow under load is measured with a dilatometer gauge placed at the other end of the beam. The dilatometer gauge reads to 0.0001 inches. The mechanical advantage of the beam is 2.6, the beam rotating through an angle of less than four minutes if the total measured flow is one percent. The eccentricity due to the rotation of the beam is assumed to have no significant effect on the flow test result. Initial flow testing was done on dispersion strengthened amalgams prepared using an alloy powder to mercury ratio of 1:1. Low compressive strengths indicated that this alloy to mercury ratio produced an amalgam that was too dry, condensing poorly and failing prematurely. However, the flow test results are

considered to be valid since the load is no more than three percent of the ultimate compressive strength of the amalgam. The alloy to mercury ratio was changed to 5:7 to obtain better condensation for the compression tests and subsequent flow test samples. A commercial amalgam with and without dispersion powder was also flow tested and the results are entered for comparison. More emphasis is placed on compression rather than flow tests in this study, even though the A. D. A. Specification does not require compression tests. The compression tests relate directly the strength to the weight ratio and particle size of the dispersion powder added. On the other hand there is some doubt about (5) the clinical significance of flow. Skinner has shown that flow is dependent upon condensation pressure, which is not specified in the A. D. A. Specifications.

Compression test specimens were prepared in the same mold as flow test specimens and were aged for one week at room temperature before breaking in a Hounsfield Tensometer. The loading rate in the compression tests was 0.1 millimeters per minute. The values entered are the averages of at least three compression tests of each particular amalgam.

Dimension change tests were made according to the A. D. A. Specifications. The dimension changes were determined with the dilatometer gauge used in the flow tests.

RESULTS AND DISCUSSION

Fig. 6 shows the effect on flow (after 21 hours) of the particle

size and amount of dispersion powder in the amalgam. All flow tests were made using an alloy to mercury ratio of 1:1 with the exception of the points labelled 1 and 2 for which the alloy to mercury ratio is 5:7. The points on the curve of Fig. 6 represent only one flow test of each particular amalgam but the results show a trend predictable by the theory of dispersion hardening; i. e. flow should decrease (strength increases) for an increase in amount of dispersion phase and decrease in particle size. Point 1 gives the flow for an amalgam with a 0.5 weight ratio of dispersion powder to standard alloy powder and an alloy to mercury ratio of 5:7. The final flow was slightly over one percent - this is a 650% decrease in flow compared to the standard amalgam prepared with a 1:1 alloy to mercury ratio. Point 2 gives the flow for an amalgam with a 0.5 weight ratio of dispersion powder to commercial alloy powder. The final flow value was 0.53% which is less than one half the flow of the commercial amalgam without the dispersion powder.

Fig. 7 shows the results of compression strength versus dispersion powder size for a weight ratio dispersion powder to standard alloy powder of 0.3 and an alloy to mercury ratio of 5:7. A very sharp increase in strength occurs when the dispersion powder size is reduced to -44 microns. Further tests are needed to establish the effect of particles less than 27 microns, but an increase in compressive strength is expected.

Fig. 8 shows the results of compressive strength versus weight

ratio of (-38 +27) micron dispersion powder to standard alloy powder.

A general increase is evident up to a weight ratio of 0.5 but late testing shows a decrease in strength for a weight ratio of 0.6. This would indicate that a weight ratio of 0.5 is the maximum for this particular size of dispersion powder and beyond this, the matrix becomes overloaded.

This indication of an overload point can also be seen in Fig. 6 for flow tests of amalgams containing (-103 +74) micron dispersion powder. Using either the standard alloy matrix or a commercial alloy matrix, the flow is decreased up to a weight ratio of 0.2, beyond which it increases again. Comparing these two overload weight ratios it is ^{evident} ~~clear~~ that the overload weight ratio increases as particle size decreases.

Fig. 8 also shows the affect of adding dispersion powder to a commercial amalgam alloy. A commercial amalgam containing a 0.5 weight ratio of dispersion powder to commercial alloy, prepared as directed by the manufacturer, showed an increase in compressive strength of 15% over the commercial amalgam without dispersion powder. With a 0.5 weight ratio of dispersion powder in the standard alloy matrix, the increase in compressive strength is about 33%.

A dimension change test of an amalgam with a 0.5 weight ratio of dispersion powder to standard alloy powder and an alloy to mercury ratio of 5:7 gave a total expansion of 0.23%. This is 0.03% greater than the allowable expansion of 0.20% specified by A.D.A. However, this test was made using the standard alloy matrix which has no particular merits other than it being the reference standard. Indications are that

the dimension change of an amalgam containing a 0.5 weight ratio of dispersion powder to the commercial alloy is within the specified maximum allowable expansion.

CONCLUSIONS

The results show that dispersion strengthening can be successfully applied to mercury amalgams. The flow is most notably decreased by adding the dispersion powder to the amalgam. For the standard alloy matrix, flow was decreased by more than 600% and the compressive strength was increased by more than 30%. The alloy to mercury ratio should be less than 1:1 to ensure proper condensation of the amalgam, otherwise premature compression failure may occur due to the presence of voids.

An investigation is now in progress to determine the clinical feasibility of dispersion strengthened amalgams.

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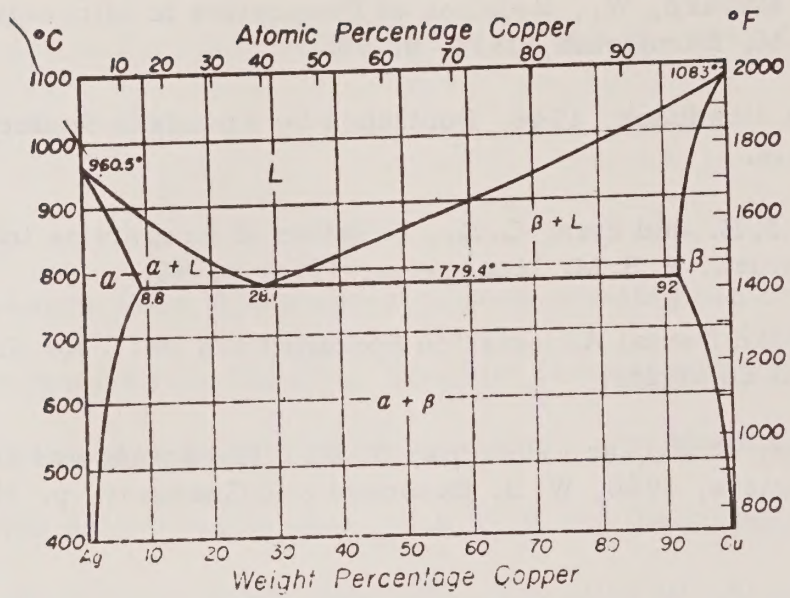


Figure 1. Silver-copper constitution diagram.

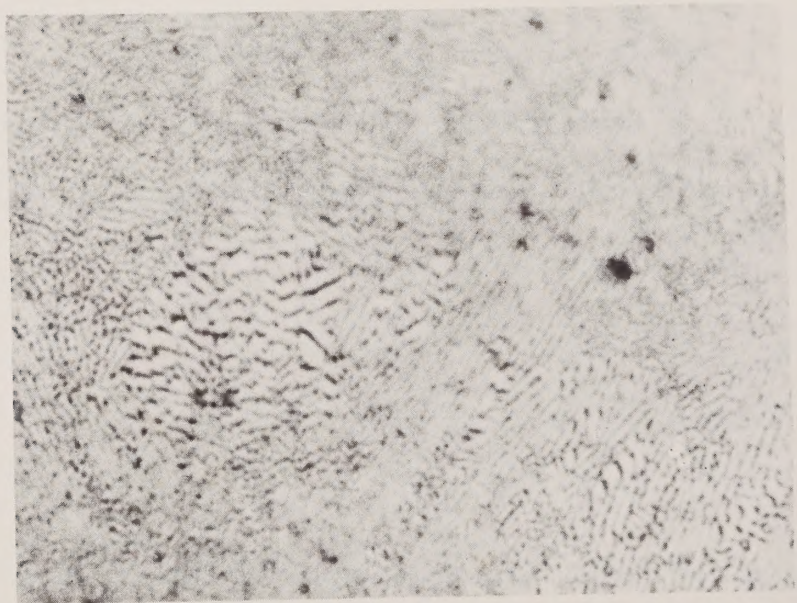


Figure 2. Silver-copper alloy microstructure x1050.

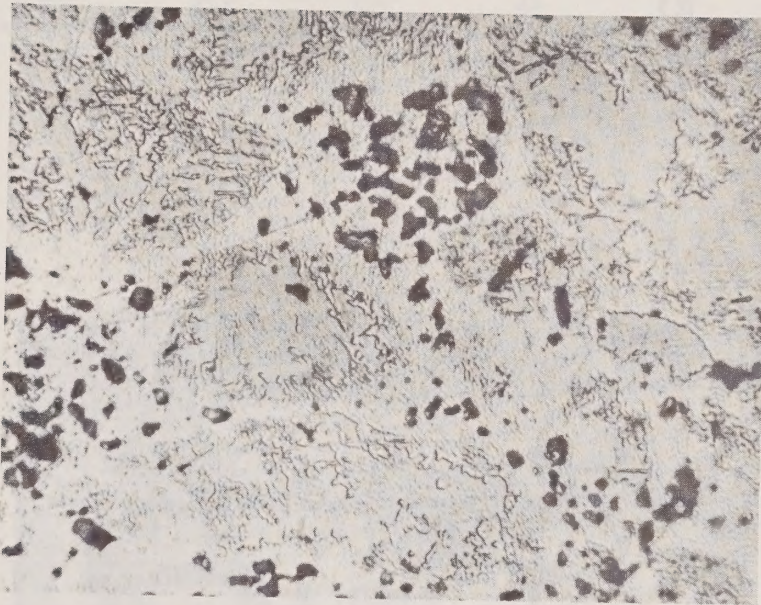


Figure 3. Standard amalgam x700.

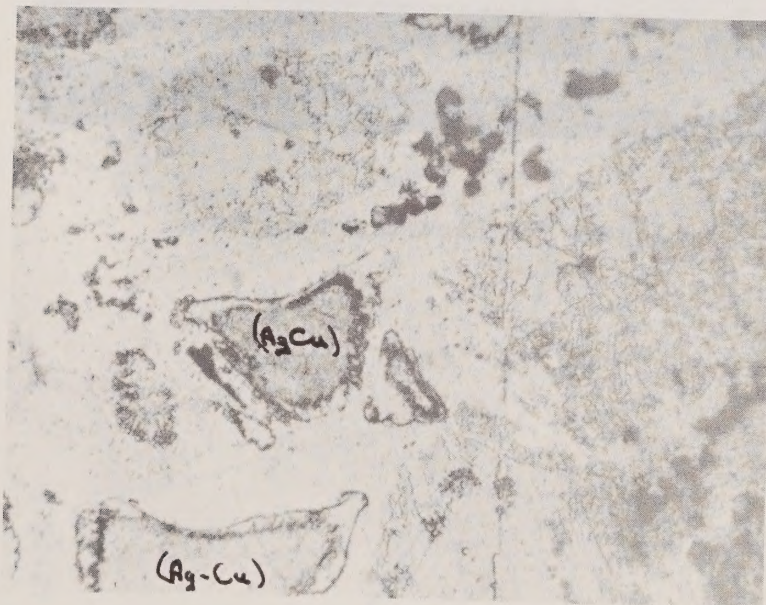


Figure 4. Dispersion strengthened amalgam x700.
The Ag-Cu particles are labelled

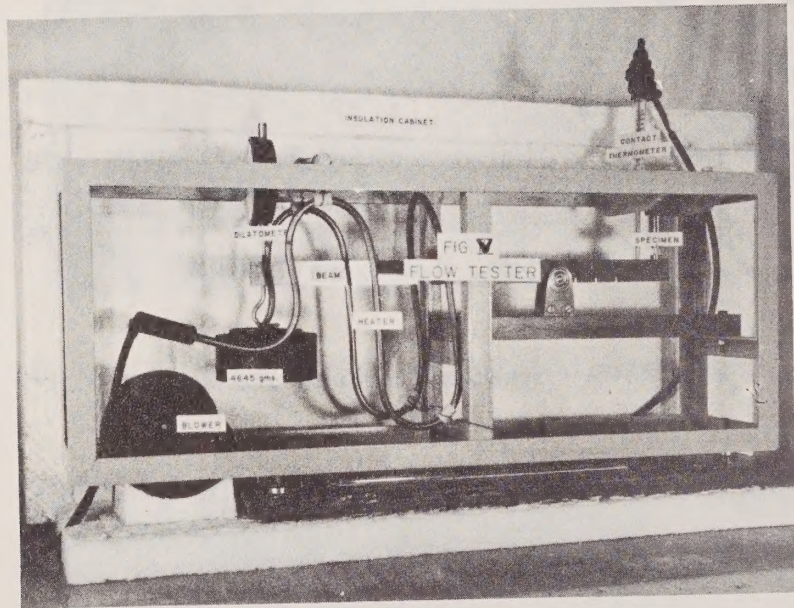


Figure 5. Flow test apparatus.

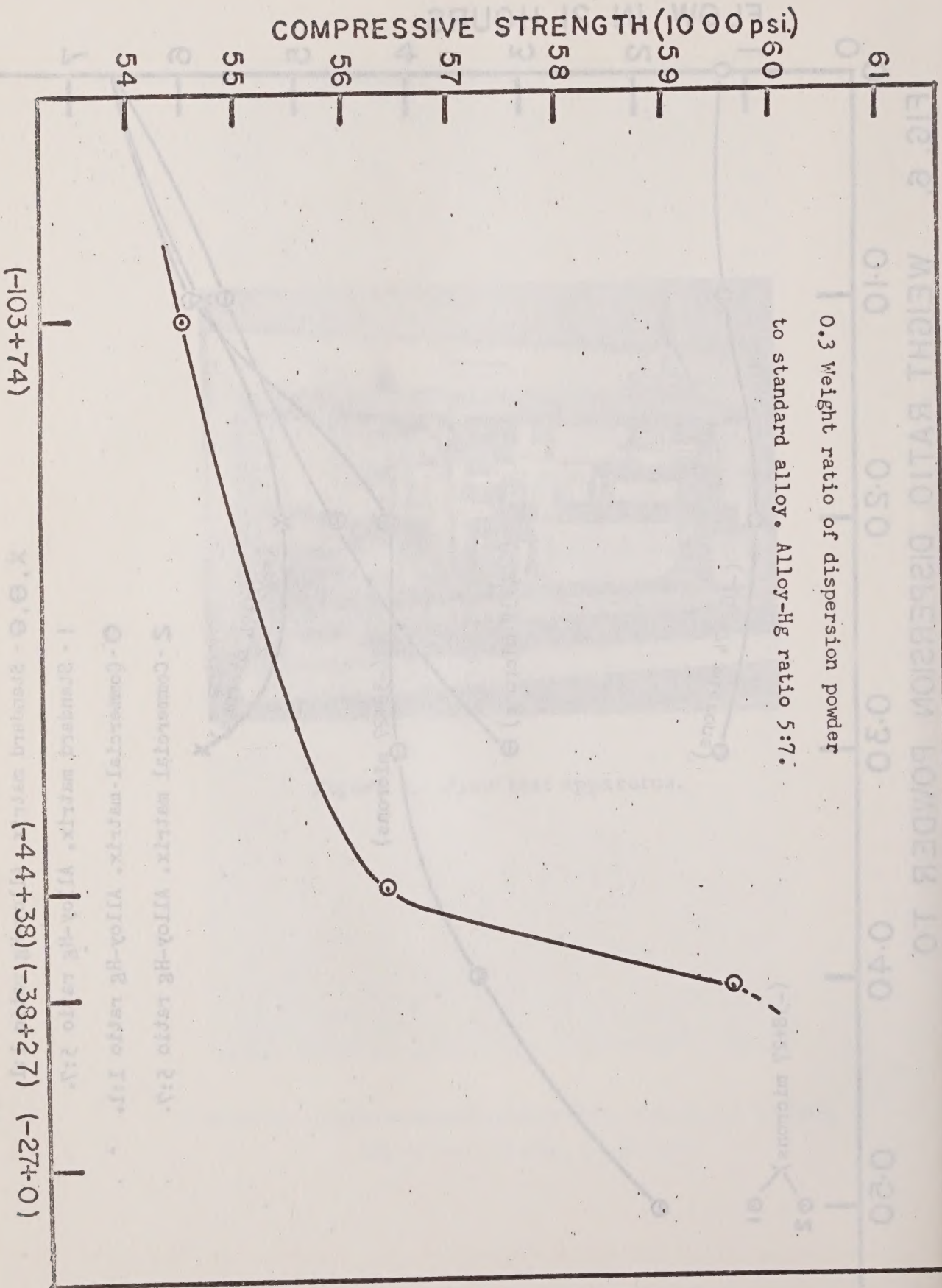
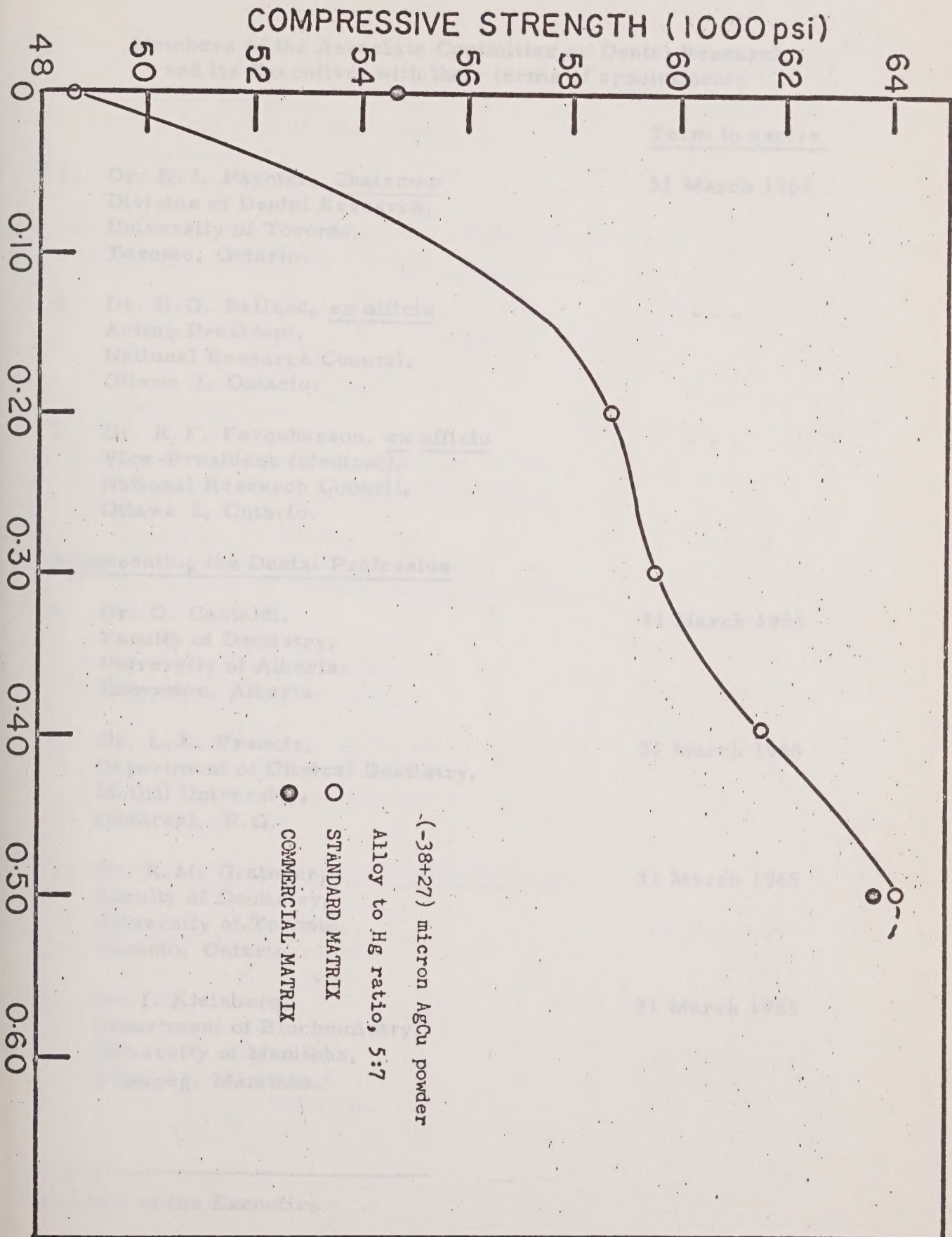


FIGURE 7. AVERAGE DISPERSION PARTICLE SIZE (MICRONS)

APPENDIX 8

FIG. 8. WEIGHT RATIO DISPERSION POWDER TO



APPENDIX S

Members of the Associate Committee on Dental Research and its Executive, with their terms of appointments

	<u>Term to expire</u>
* 1. Dr. K. J. Paynter, <u>Chairman</u> Division of Dental Research, University of Toronto, Toronto, Ontario.	31 March 1963
2. Dr. B. G. Ballard, <u>ex officio</u> Acting President, National Research Council, Ottawa 2, Ontario.	- - -
3. Dr. R. F. Farquharson, <u>ex officio</u> Vice-President (Medical), National Research Council, Ottawa 2, Ontario.	- - -
<u>Representing the Dental Profession</u>	
4. Dr. C. Castaldi, Faculty of Dentistry, University of Alberta, Edmonton, Alberta.	31 March 1963
5. Dr. L. E. Francis, Department of Clinical Dentistry, McGill University, Montreal, P.Q.	31 March 1965
6. Dr. R. M. Grainger, Faculty of Dentistry, University of Toronto, Toronto, Ontario.	31 March 1965
* 7. Dr. I. Kleinberg, Department of Biochemistry, University of Manitoba, Winnipeg, Manitoba.	31 March 1965

* Member of the Executive

Term to expire

8. Dr. G. Nikiforuk,
Division of Dental Research,
University of Toronto,
Toronto, Ontario.

31 March 1963

Representing the Royal Canadian Dental Corps,
Department of National Defence

9. Brig. K.M. Baird, ex officio
Director General of Dental Services,
Royal Canadian Dental Corps,
Department of National Defence,
Ottawa, Ontario.

- - -

Representing the Division of Dental Health,
Department of National Health and Welfare

10. Dr. H.K. Brown, ex officio
Chief, Dental Health Division,
Department of National Health and Welfare,
Ottawa, Ontario.

- - -

Representing Dental Research,
Department of Veterans Affairs

11. Dr. L.A. Kilburn, ex officio
Director of Dental Research,
Department of Veterans Affairs,
Ottawa, Ontario.

- - -

Representing the Basic and Medical Sciences

12. Dr. L.F. Belanger,
Department of Histology,
University of Ottawa,
Ottawa, Ontario.

31 March 1965

- * 13. Dr. J.P. Lussier,
Faculty of Dentistry,
University of Montreal,
Montreal, P.Q.

31 March 1964

* Member of the Executive

INITIAL DISTRIBUTION

Term to expire

- | | |
|---|---------------|
| 14. Dr. A.G. Gornall,
Department of Pathological Chemistry,
University of Toronto,
Toronto, Ontario. | 31 March 1965 |
| 15. Dr. W.C. Wilt,
Department of Bacteriology,
Medical College,
University of Manitoba,
Winnipeg, Manitoba. | 31 March 1965 |
| 16. Dr. C.P. Leblond,
Department of Anatomy,
McGill University,
Montreal, P.Q. | 31 March 1963 |
| 17. Dr. A.J. Rhodes,
School of Hygiene,
University of Toronto,
Toronto, Ontario. | 31 March 1963 |
| 18. Dr. R.L. de C.H. Saunders,
Department of Anatomy,
Dalhousie University,
Halifax, Nova Scotia. | 31 March 1964 |
| * 19. Dr. G.D. Scott,
Department of Physics,
University of Toronto,
Toronto, Ontario. | 31 March 1963 |
| 20. Mr. A.D. Armstrong, <u>Secretary</u>
National Research Council,
Ottawa, Ontario. | |

* Member of the Executive

INITIAL DISTRIBUTION

(i) To members of the Associate Committee on Dental Research

- | | |
|--------------------------------------|-------------------------------|
| 1. Dr. K.J. Paynter, <u>Chairman</u> | 11. Dr. L.A. Kilburn |
| 2. Brigadier K.M. Baird | 12. Dr. I. Kleinberg |
| 3. Dr. B.G. Ballard | 13. Dr. C.P. Leblond |
| 4. Dr. L.F. Belanger | 14. Dr. J.P. Lussier |
| 5. Dr. H.K. Brown | 15. Dr. G. Nikiforuk |
| 6. Dr. C. Castaldi | 16. Dr. A.J. Rhodes |
| 7. Dr. R.F. Farquharson | 17. Dr. R.L. de C.H. Saunders |
| 8. Dr. L.E. Francis | 18. Dr. G.D. Scott |
| 9. Dr. A.G. Gornall | 19. Dr. W.C. Wilt |
| 10. Dr. R.M. Grainger | 20. Mr. A.D. Armstrong |

(ii) To other persons:

21-26 Deans of Dental Schools (who are not members of the Committee)

- | | | |
|----------------------|---|-------------------|
| 21. Alberta | - | Dr. H.R. MacLean |
| 22. British Columbia | - | Dr. S. Wah Leung |
| 23. Dalhousie | - | Dr. J.D. McLean |
| 24. McGill | - | Dr. J. McCutcheon |
| 25. Montreal | - | Dr. P. Geoffrion |
| 26. Toronto | - | Dr. R.G. Ellis |
| 27. Board Room Copy | | |
| 28. Library | | |
| 29-35 Reserve | | |

ASSOCIATE COMMITTEE ON DENTAL RESEARCH

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1. The first of the great principles of the Constitution is the separation of powers.

2. The second is the system of checks and balances.

3. The third is the federal principle.

4. The fourth is the principle of the right of the people to alter or to abolish the government.

5. The fifth is the principle of the right of the people to be secure in their persons, houses, papers, and effects.

6. The sixth is the principle of the right of the people to a fair trial by jury.

7. The seventh is the principle of the right of the people to be free from the oppression of the government.

8. The eighth is the principle of the right of the people to be free from the oppression of the government.

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NATIONAL RESEARCH COUNCIL

Proceedings

of the

Thirtieth Meeting

of the

ASSOCIATE COMMITTEE ON DENTAL RESEARCH

5 - 6 March 1964

Introduction

The first session convened in the Council Chamber of the National Research Council, Ottawa, at 9:30 a.m., 5 March 1964.

1. Attendance

Dr. J-P. Lussier, Chairman

Dr. L.F. Belanger

Dr. R.V. Blackmore

Dr. R.C. Connor

Dr. R.F. Farquharson

Dr. L.E. Francis

Dr. A.G. Gornall

Dr. R.M. Grainger

Col. D.E. Hillier

Dr. A.M. Hunt

Dr. I. Kleinberg

Dr. C.P. Leblond

Dr. S.Wah Leung

Dr. M. Poplove

Dr. A.J. Rhodes

Dr. R.L. de C.H. Saunders

Dr. W.C. Wilt

Mr. A.D. Armstrong, Secretary

Mrs. J.A. Van Laer, Acting Secretary

Regrets at their inability to attend were received from

Dr. G. Nikiforuk

Dr. D.M. Tanner (Dept. of Veterans' Affairs)

Brig. K.M. Baird (Dept. of National Defence)

The Chairman extended a welcome to the new members of the Committee and guests, introducing Colonel D.E. Hillier who attended in place of Brigadier K.M. Baird; and Dr. R.C. Connor of the Department of National Health and Welfare.

2. Minutes of the Twenty-Ninth Meeting

The Minutes of the Twenty-Ninth Meeting had been distributed to all members and were therefore taken as read.

It was MOVED by Dr. A.G. Gornall and SECONDED by Dr. R.M. Grainger

THAT the Minutes be approved.

CARRIED

3. Chairman's Remarks

Introduction

Before I bring to your attention some of the points which need clarification for better conduct of the business of this Committee, I would like to pay tribute to my predecessor at the chair, Dr. K.J. Paynter, who did so efficiently exert his sense of leadership as Chairman of this Committee between 1959 and 1963. Thanks to his tireless efforts, the Canadian dental research workers have experienced better working conditions and benefited from the augmented prestige dental research has gained in scientific and professional milieux. It would be over-ambitious on my part to try to duplicate what he did. I only expect with your help to keep on the beam striving for greater altitude.

I would also like to make you aware that this Committee is starting afresh, not only with a new Chairman but with a new Secretary and two new members in the Executive, notwithstanding the two new appointees previously introduced to you. I hope that this transfusion will bring no acute reactions and that antibodies will build up very slowly. The only inconvenience is that some matters may have been slowed down temporarily, but the normal momentum should be reached soon.

i Leadership

This Committee has implicitly accepted the role of a research sponsor, coordinator and integrator. This means that the Committee is not only distributing the money made available from public funds for dental research, but is also setting forth the policies which guarantee their best use. Hence the principle accepted last year that the Committee should back the project and not only the man. Hence also our preoccupation to consider clinical research within the realm of activities which should be encouraged by the Committee.

It came to my mind, following a release of information which was directed to me last summer, that there are areas of research which are not strongly enough investigated by our research people. Dental caries, through its economical

and epidemiological importance, should receive much greater attention. In addition, studies such as metabolic aspects of fluoride are of utmost importance, and still very few workers are attracted on these lines. Perhaps we have reached the point where a conference should be held in order to determine how our efforts can best be coordinated. This topic could well be considered at a future Canadian Conference on Dental Research.

ii The Glassco Report highlights from the standpoint of Medical Research

In the chapter on a "National Scientific Policy" there are statements which should be brought to the attention of the members of this Committee, as they will influence the future policies governing the support of research by federal agencies.

"It is not unfair to say that the scientific policy of Canada today is the result, rather than the cause of growth in the many scientific activities undertaken by government.

Criteria in the formulation of policies

The determination of what Canada can afford to spend or what it cannot afford to refrain from spending involves consideration of a number of different factors. The selection of the fields of research in which effort is to be applied should take into account the economic needs of the country, its geography, its industrial plant and capacity, the skill of its peoples and the education, all facilities available for their enhancement..."

"The degree of effort to be expended in each field calls for the establishment of priorities and a careful weighing of the claims of each for support against the potential economic and social value of the scientific gains which may result. Here the questions arising may be of the greatest difficulty. For example, is the challenge of Canada's high rate of infant mortality sufficient to divert research funds from, say, marine biology? Does the expenditure of but five per cent of the research budget on human life and health represent a reasonable apportionment?"

Following this statement, we can contend that the spending of less than \$400,000 for dental research in comparison to the \$100,000,000 spent annually by the public for dental services may be looked at as falsely adjusted when health research is considered top priority among the areas where the government is granting support.

In 1961-62, there was 11 million dollars granted by the Federal Government for medical research. Assuming that expenses for medical and hospital sciences could have reached the one billion dollar mark, the ratio would still be 1 to 100; absolutely and relatively, dental research is way behind.

The commissioners stated that this amount of 11 million was only 4.5% of what the Federal Government is spending for research. This proportion should be substantially increased, and the conditions offered to medical research people should be improved, in order to prevent the exodus of Canadian scientists to U.S. institutions.

As a matter of principle medical research should be carried on in universities and hospitals. The Glassco Report is definitely backing the Farquharson programme which is requesting 25 million dollars per year for medical research.

iii Inquiries in dental schools

If for a moment we forget about these hopeful recommendations for the Glassco Report, to look at the answers supplied by the dental deans, we will find ourselves facing a demanding future. The deans of Canadian dental schools were solicited three times to supply information on the future needs for dental research, three times between May and September 1963. In May, I sent a questionnaire to the deans on account of a meeting to be held in Toronto, where they were invited to meet with the representatives of granting bodies.

I gathered the following information which is described at length in the report distributed this morning.

In June, Dr. Spinks' Forecasting Committee sent a questionnaire to heads of departments of all Canadian universities requesting information about future needs with respect to research projects. The results of this survey revealed that Canadian dental schools will need one million dollars by 1968. These estimates are much more conservative than the ones supplied to Mr. Armstrong one month later. In this last survey the deans indicated a need for two million dollars by 1968. This is symptomatic of a situation where imagination as well as basic facts are called in to set figures. It is obvious that the deans were becoming more ambitious every month or that, as they become more bored, they felt more hungry. It is also obvious that the figures are groping along a steep ladder and that a certain height will have to be reached within a short time.

If we look at the previsions for this year, \$490,800, we find that the amount requested in the applications is not so far behind (\$415,000).

I believe we have now sufficient elements to introduce into a memorandum which can be directed to the proper authorities, in order to call for a mobilization of the necessary resources and place dental research at the right level of emphasis in relation to the other fields of research.

iv Relations of N.R.C. and dental schools

There was a motion in last year's Proceedings requesting that a case be prepared in order to favor initiation by NRC of a more direct type of support to dental schools by grants to deans.

I must apologize for not having done so. At the psychological moment to intervene, I received a recommendation from the granting Committee for allotments to our Committee which was much lower than the sum stipulated in our own request for this year. I realized it was not appropriate under such circumstances to make a case for an innovation when the features of the regular pattern could not even get their share. In preference to this, I presented a memorandum to legitimate the amount introduced in our motion concerning the 1964 budget.

However, it should be appropriate following this meeting to take action on this matter, after proper consultation with the deans. For the same reasons, it would be advisable that a member of the Executive be delegated to the regional meeting of dental schools and the ADA Council on Research held simultaneously in Chicago or New York. Good use could be made of the previous experience of the National Institute of Dental Research with the Deans' grants in the American dental schools.

v The problem of intercommunication between dental research workers in Canada is still unsolved. Until we come to a suitable formula, I wonder if it would not be advisable to set up another Research Conference similar to the previous ones which were held in Toronto in 1961 and Banff in 1962. The Council could act as a sponsor with another agency, in order to cover the expenses involved in such a conference.

vi I.M.R.C.C.

As Chairman of this Committee, I had the privilege to attend the annual meeting of the I.M.R.C.C. last January. During the sessions several points were raised and solutions were proposed which may be of some help to this Committee.

a) Professional Assistance

Dr. Farquharson made it clear that the Medical Council is revising some of its policies concerning the training of the medical research personnel and the use

of professional assistants. I shall call later upon Dr. Farquharson to enlighten us on the effects of the new decisions taken by MRC.

b) Referees.

It seems that the screening of grant applications has loopholes which are creating uncomfortable situations in all organizations. Among the policies enforced by some agencies which may be of some merit to us is the following: a panel of referees is made up of specialists of same in different disciplines but who were all familiar with the research done in a particular area, e.g. cancer, growth, nutrition. They meet at a definite time and proceed through the screening.

Under such circumstances the Committee would not be in a position to appoint a group of individuals to meet and act as the one discussed. But we could recall that referees may be appointed in a specific field such as Orthodontics, Growth and Development, Calcification Dental Histology, Anthropology and Genetics, Oral Biology and Pathology, Dental Materials, etc. This would require that new applicants send in applications at least one or two months earlier than at present.

Leaving the Chairman with the responsibility of selecting referees places him in a very delicate situation.

The decision is left to the Committee to propose a suitable mechanism. The one we actually use has many weak points. Under the same headline of referrals, we should consider if it is appropriate to communicate to the applicants the comments and statements made by referees. Although there are many suspected inconveniences in doing so, applicants may benefit from this information for future use.

c) Grant renewal.

Another point which is worthy of mention is the following policy regarding grant renewal. As a matter of fact, some agencies after granting for one year, renew automatically for the second year. Before renewing for a third year, they look seriously at the results and according to their value decide to forsake the support or to continue it. They may also on the third year make suggestions in order to encourage the use of different methods or to indicate more effective orientation.

d) Travel allowances.

I also learned at this meeting that MRC is implementing its policy to allow travel expenses on the basis of

\$150/5000 in N.B., Que., and Ont., \$200/5000 in N.S., Manitoba and Sask., and \$250/5000 in Alberta and B.C.

vii There are two points I would like finally to bring to your attention; one is related to the deadline for sending in applications, the other is the necessity of holding an Executive Meeting in the fall (September or October). It would help to carry the business matters more quickly if these two points could be taken into consideration.

viii Attention was drawn to the "Report of the meeting of deans of dental schools and representatives of dental research sponsoring agencies" as follows:

REPORT OF THE MEETING OF DEANS OF DENTAL SCHOOLS AND
REPRESENTATIVES OF DENTAL RESEARCH SPONSORING AGENCIES

Drs. R. Ellis, Hector MacLean, S. Wah Leung, J. Neilson, J.P. Lussier, H.K. Brown and K.J. Paynter met, May 18, 1963, in Toronto to establish the needs to promote and foster dental research in dental schools of Canada.

Previous to the meeting, a questionnaire was sent to the deans of the dental schools and the following information was secured about actual staff and future enrollment, fields of research, facilities, technical assistance, equipment and supplies. The questionnaire was sent to the seven deans; six answers were expected but five only were returned.

a) Actual Staff

From the answers it could be established that there are about fifty people actively engaged in dental research in Canada. This includes not only people in the dental schools but also those from other departments of the universities interested in dental research. It was asked if there were some people in departments other than those of dental schools who could direct or participate in some dental research projects. The questions did not receive a direct answer, although it is clear that some participation could be available at this time in these departments.

b) Future Enrollment

The deans were asked how many graduates are needed in the next five years to fill appointments in the fields where dental research is considered mandatory. The response indicated a need for from five to thirteen in each school; as a whole, forty graduates should receive training in order to perform and direct dental research in the next five years. The need is much greater in the basic sciences; at least thirty out of these forty people will be oriented in the basic sciences.

c) Fields of Research

The disciplines into which dental research is actually carried are as follows:

1. basic sciences: anatomy, anthropology, biochemistry, bacteriology, histology, nutrition, pathology, pharmacology, physiology, radiobiology.
2. clinical areas: dental materials, operative dentistry, orthodontics, pedodontics, periodontics and prosthodontics.

d) Facilities

Two dental schools actually can offer ten thousand square feet for the purpose of dental research. Three schools offer about thirteen thousand square feet; in departments other than dental schools, five thousand square feet are available: all together, barely thirty thousand square feet. It is not expected in the next five years to provide additional facilities including more than fifteen thousand square feet. This does not take account of the facilities available in the new schools which may come into operation during this period. Under such circumstances, floor space may become a limiting factor in the development of dental research, unless dental schools or universities are willing to make a special effort to provide more facilities. This point needs further clarification as there may have been underestimation of future availabilities.

e) Technical Assistance

Three schools out of six are providing technical assistance to their research staff. They are supporting on their own budget, fully or partly, twenty-three technicians. It would be interesting to know now how many are provided through grants in aid, in order that the ratio of technical assistance to research manpower could be learned.

f) Equipment

To the question concerning equipment needed for purposes of dental research, two deans replied that they expected to buy on their research budget equipment amounting to seventy-five thousand dollars in the next five years. Others did not attempt to estimate any figure. In three schools the research equipment needed for the next five years is over one quarter of a million dollars. Other deans did not specify. From the given answers, it was established that for all six schools about seventy-five thousand dollars per year would be needed in equipment.

g) Supplies

From the answers received, the opinion was unanimous that research workers need a strict minimum of a hundred dollars a month in order to carry on their research projects properly.

Summary

If it is foreseen that the actual research staff of about forty people will increase to eighty within the next five years. Technical assistance will have to more than double in order to supersede the ratio of one to one in terms of technical aids to research workers. Space may soon become an acute problem for the expansion of dental research in the dental schools. Close to four hundred thousand dollars will have to be spent in equipment within the next five years; two-thirds of this amount will have to be sought outside of the universities' budgets.

A discussion followed regarding means of promoting increased support of dental research in Canada.

4. Financial Report

(a) Status of the Committee

The Committee was informed that up to \$170,000 could be made available to the Committee for 1964-65 grants; \$45,000 (approximately) for Fellowships; \$18,500 (approximately) for Scholarships; \$28,500 (approximately) for Associateships; (the amounts for Scholarships and Associateships include research grants awarded during the first year of tenure of these awards) \$2,500 for Committee expenses.

Of the \$170,000 for grants, \$26,000 was encumbered for term grants previously approved.

5. Medical Research Council

Dr. R.F. Farquharson outlined the new policies of the Medical Research Council and the problems encountered in supporting medical research. He pointed out that the limited funds available permitted the Medical Research Council to grant an even smaller percentage of requests than the Associate Committee on Dental Research.

The Chairman noted that Dr. Farquharson's remarks confirmed the need for the Committee, in conjunction with the Deans of Canadian Dental Schools, to decide upon a future policy with regard to support of dental research in Canada.

6. Executive Action 1963-64

The Chairman drew attention to the following Executive action taken since the last meeting.

Fellowships

- Gardner, D.G. Awarded a Graduate Dental Research Fellowship to work in the Department of Oral Diagnosis, at Indiana University.
Value - \$4,000
- Gray, A.S. Declined a Graduate Dental Research Fellowship to work in the Faculty of Dentistry, University of Toronto under the supervision of Dr. R.M. Grainger.

Grants in Aid of Research

- Nikiforuk, G. DT-84
Dentistry Requested permission to use \$1,500 of his
Toronto operating grant funds to assist in research
 work while on sabbatical leave at the
 Universities of Copenhagen and Lund.
 Request Not Granted.
- Nikiforuk, G. Senior Research Fellowship Requested.
 In connection with sabbatical leave, to
 attend the University of Copenhagen, and
 to visit Sweden and Belgium.
 Requested - \$600
 Awarded a Travel Grant (\$600) as a
 supplement to Travel Grant (\$1,200) awarded
 in March, 1963.
- Sinclair-Hall, Operating Grant Cancelled.
A.H. DA-92 \$1,500 Polyvinyl-Alcohol Sponge and
Anatomy the Healing of Bony Defects.
Manitoba Refunded \$1,500 plus \$388.29 unspent
 balance of 1962-63 grant.
- Trott, J.R. Travel Grant Requested.
Periodontology \$355 to attend the meeting of the International
Manitoba Association for Dental Research, Los Angeles,
 U.S.A., 19-22 March, 1964.
 Travel Grant not approved.

7. Revisions in Regulations

The Committee noted that the Council had not approved a change in regulations to permit consideration of non-Canadian, non-residents of Canada for Dental Research Scholarships and Associateships. The Council had agreed, however, that in special cases, such applicants could be considered.

8. Meeting of the Executive

The Chairman informed the Committee that the members of the Executive (Dr. L.E. Francis, Dr. A.G. Gornall, and Dr. I. Kleinberg, in addition to the Chairman) had met on 4 March, 1964 at 9:30 a.m. Preliminary consideration had been given to applications for Fellowships, Scholarships, Associateships, and Grants; the recommendations of the Executive were submitted to the Committee as each application was considered.

9. Applications for Fellowships

Fourteen applications for Dental Research Fellowships tenable during 1964-65 were considered: five for renewal of current Fellowships, and nine for a first award. Action was taken as follows:

Ambrozaitis, J.	To work in the Dept. of Bacteriology, University of Montreal under Dr. G. Nogrady	APPROVED \$4,000
Ellis, W.B.	To work in the Dept. of Biochemistry, University of Toronto under Dr. C.S. Hanes	APPLICATION WITHDRAWN
Gardner, D.G.	To work in the Dept. of Oral Diagnosis and Oral Medicine, Indiana University under Dr. D.F. Mitchell	APPROVED \$4,500
Golub, L.M.	To work in the Dept. of Oral Biology, University of Manitoba under Dr. C.M. Dowse	APPROVED \$3,500
Haselton, R.D.	To work in the School of Dentistry, University of Toronto under Dr. K.J. Paynter	APPROVED \$3,500
Houde, M.M.	To work in the Dept. of Pathology, University of Michigan under Dr. D.A. Kerr	FELLOWSHIP DECLINED
Kaufman, H.W.	To work in the Dept. of Oral Biochemistry, University of Manitoba under Dr. I. Kleinberg	APPROVED \$4,500
Lewis, D.W.	To work in the Faculty of Dentistry, University of Toronto under Dr. R.M. Grainger	APPROVED \$3,500
Nichol, N.E.	To work in the Dept. of Oral Biology, University of Manitoba under Dr. E.T. Pritchard	APPROVED \$3,500

Sandham, H.J.	To work in the Dept. of Oral Biology, University of Manitoba under Dr. I. Kleinberg	APPROVED \$5,000
Taichman, N.S.	To work in the Dept. of Pathology, Banting Institute, University of Toronto under Dr. H.Z. Movat	APPROVED \$5,500
Weinstock, A.	To work at the Harvard School of Dental Medicine under Dr. J.T. Irving	APPROVED \$4,000
Willinsky, M.	To work in the Dept. of Microbiology, University of Toronto, under Dr. A. Rhodes	NOT APPROVED
Windsor, C.L.	To work in the Dept. of Anatomy, University of Alberta under Dr. T.S. Leeson	APPROVED \$4,000

The Committee decided that due to lack of funds the allowance of \$500 (recommended Proc. 28th Meeting, p.13) to be paid to married Fellows without children be waived for 1964-65. Married Fellows with children however, would be allowed an appropriate allowance.

A special case was made for Dr. C.L. Windsor who was beyond the age limit for Dental Fellows. The Committee agreed to award a Fellowship for one year to Dr. Windsor, with the specification that in future years he find other means of support.

It was MOVED by Dr. I. Kleinberg and SECONDED by Dr. A.M. Hunt,
THAT payment of these grants be made as outlined in the above schedule.

CARRIED

10. Applications for Scholarships

One application for a Dental Research Scholarship tenable during 1964 to 1967 was considered. Action was taken as follows:

Burgess, R.C.	To work in the Faculty of Dentistry, University of Toronto	APPROVED \$8,500 plus \$5,000 Operating Grant, plus payment of applicant's share of pension and other benefits in which university staff members normally participate.
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It was MOVED by Dr. C.P. Leblond and SECONDED by Dr. I. Kleinberg THAT the payment of this award be made as outlined above.

CARRIED

11. Applications for Associateships

The Committee discussed at length the procedure for renewal of Associateships.

It was MOVED by Dr. C.P. Leblond and SECONDED by Dr. L.E. Francis THAT the Executive be empowered to set up a Committee to establish a procedure to evaluate the performance of an Associate, following his initial period of tenure, in order that a thorough assessment of the Associate in question be made before renewal of his award.

CARRIED

E.T. Pritchard

The Committee agreed that, in view of the above new policy, the initial appointment of Dr. E.T. Pritchard be extended for one more year. An application for renewal of his appointment would be thoroughly reviewed by the special Committee.

The salary of the incumbent holding the Dental Research Associateship tenable during 1964-65 was considered. Action was taken as follows:

E.T. Pritchard	1963-64		1964-65
Dept. of Oral Biology		Recommended	Approved
Faculty of Dentistry	\$9,600*	\$10,300*	\$10,300*
University of Manitoba			

*Plus payment of applicant's share of cost of pension and other benefits in which university staff members normally participate.

Two new applications for Associateships were then considered. Action was taken as follows:

Goldner, M.	To work in the Division of Dental Research, Faculty of Dentistry, University of Toronto	APPROVED \$11,500 plus \$5,000 Operating Grant plus payment of applicant's share of cost of pension and other benefits in which university staff members normally participate.
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Melcher, A.H.	To work in the Faculty of Dentistry, University of Alberta	APPLICATION WITHDRAWN
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It was MOVED by Dr. R.M. Grainger and SECONDED by Dr. R.C. Connor THAT payments be made for Scholarships and Associateships as outlined above.

CARRIED

12. Applications for Grants 1964-65

The Chairman noted that funds were already committed for the coming year for Term Grants as follows:

Francis, L.E. Pharmacology & Therapeutics McGill	Investigation of a gingival histamine and serotonin antagonist DT-76	\$5,000 The second Instalment of a three-year Term Grant
Hunt, A.M. Dentistry Toronto	Measurement of strontium ⁹⁰ and carbon ¹⁴ in primary teeth of Canadian children DT-82	\$8,000 The second Instalment of a three-year Term Grant
Leblond, C.P. Anatomy McGill	Entry of arbohyrates and proteins into teeth: formation of matrix of enamel and dentine DT-23	\$5,000 The third Instalment of a three-year Term Grant
Paynter, K.J. Dental Research Toronto	Factors influencing tooth morphology and caries susceptibility DT-72	\$8,000 The third Instalment of a three-year Term Grant

Thirty applications for Operating Grants, six for grants to support Summer Undergraduate Assistants, and five for Major Equipment Grants, were then considered. These had been reviewed before the meeting by scientists qualified in the particular field involved, and copies of the reviewers' comments were made available.

Name

<u>Department</u> <u>University</u>	<u>Project Title</u>	<u>Requested</u>	<u>Approved</u>
Baril, C. Orthodontics Montreal	Refinement of dental electromyography DA-107	\$6,035	Recommended \$6,000

<u>Name</u> <u>Department</u> <u>University</u>	<u>Project Title</u>	<u>Requested</u>	<u>Approved</u>
Blackmore, R.V. Dental Research Alberta (Edmonton)	An investigation of the factors influencing the propagation of Herpesvirus hominis in animal cell cultures and in experimental animal infections DA-102	\$7,630	Recommended \$3,500 \$7,300
Castaldi, C.R. Dentistry Alberta (Edmonton)	The influence of water borne fluorides on human tooth morphology DA-121	\$4,222	Recommended \$2,200
Copp, D.H. Physiology Br. Columbia	Phosphorus and calcium deficiency: effects on bones and teeth DT-59	\$7,200	Recommended as a three-year Term Grant \$7,000 per year
Dale, J.G. Dental Research Toronto	The study of bone resorption by implantation and autoradiographic methods DA-99	\$5,600	Recommended \$3,000
Dawes, C. Physiology Manitoba	Factors influencing the secretion and precipitation of salivary proteins DA-122	\$8,530	Recommended \$3,500
Donefer, E. Animal Science Macdonald College McGill	Studies on fluorine metabolism	\$4,000	Not recommended
Dowse, C.M. Oral Biology Manitoba	Metabolism of periosteum DA-81	\$13,740	Recommended \$8,000
Edmund, A.G. Royal Ontario Museum Toronto	Dynamics of tooth replacement processes in modern reptiles DA-104	\$5,650	Recommended \$5,000
Grainger, R.M. Dental Research Toronto	Statistical consultation and analysis unit DA-123	\$10,935	Recommended \$4,000

<u>Name</u> <u>Department</u> <u>University</u>	<u>Project Title</u>	<u>Requested</u>	<u>Approved</u>
Hamilton, I.R. Oral Biology Manitoba	Anaerobic carbohy- drate metabolism by Streptococcus salivarius DA-124	\$7,908	Recommended \$3,500
Hunt, A.M. Dental Research Toronto	Collagenolytic activity in periodontal structures	\$5,110	WITHDRAWN
Johnston, M.C. Dental Research Toronto	Studies on the early embryological development of the face DA-125	\$8,850	Recommended \$6,500
Jordan, R.E. Operative Dentistry Dalhousie	Human primary molar development DA-106	\$3,300	Recommended \$1,000
Kasloff, Z. Restorative Dentistry Manitoba	Metallographic studies of soldered joints and cast gold parent metals DA-107	\$7,895	Recommended \$5,000
Khairat, O. Bacteriology & Immunology Manitoba	The prevention of dental post-extraction bacteremia DA-101	\$4,339	Recommended \$3,800
Kleinberg, I. Biochemistry Manitoba	1. Metabolism of dental plaque 2. The effect of refinement of dietary carbohydrate on dental caries DT-78	\$15,800	Recommended as a three-year Term Grant \$12,500 per year
Little, J.S. Dentistry Alberta (Edmonton)	An investigation of the possible signi- ficance of the deve- loping vascularity in the palatal tissues during normal and abnormal closure of the palate DA-126	\$1,515	Recommended \$1,200
Lussier, J.-P. & Lague, J.-G. Dental Surgery Montreal	Antagonism of flu- orine-citrate in the calcifying tissues under conditions where blood citrate is increased DA-70	\$6,915	Recommended \$5,800

Name Department <u>University</u>	<u>Project Title</u>	<u>Requested</u>	<u>Approved</u>
Mezl, Z. Oral Pathology Montreal	Experimental modifica- tions of the perme- ability of the superficial zone of enamel caries DA-79	\$2,100	Recommended \$1,600
Popovitch, F. Dentistry Toronto	Relation of oral habits to facial development	\$3,200	Not recommended
Pritchard, E.T. Oral Biology Manitoba	Sulfolipids of rat tissues DA-109	\$11,100	Recommended \$7,500
Roberts, M. Orthodontics Toronto	Simultaneous electro- myographic and cephalometric radio- graphic studies of mandibular positions	\$3,467	Not recommended
Saunders, R.L. de C.H. Anatomy & Histology Dalhousie	Microangiographic studies of the monkey and human peridental vessels DA-105	\$3,418	Recommended \$3,000
Simard-Savoie, S. Pharmacology Montreal	Experimental and clinical studies of the analgesic activity of phenothiazine derivatives and other new analgesics Da-127	\$6,625	Recommended \$3,000
Spouge, J.D. Oral Biology Br. Columbia	Hypersensitivity reactions in mucous membranes and 3 other minor projects DA-93	\$1,850	Recommended \$1,000
Trott, J.L. Periodontology Manitoba	An investigation into the incidence and aetiology of chronic hyperplastic gingivitis and localised gingival recession in adolescent DA-128	\$800	Recommended \$400

Name Department University	Project Title	Requested	Approved
Tuba, J. Biochemistry Dentistry Alberta (Edmonton)	A. The influence of intestinal absorption on body fluids B. Mucoproteins in gingival tissue in health and in disease DA-94	\$8,310	Recommended \$7,000
Woodside, D.G. Orthodontics Toronto	The relations between human mandibular growth rate and form DA-110	\$4,415	Recommended \$3,500
Youdelis, W.V. Mining & Metallurgy Alberta (Edmonton)	Metallurgical fundamentals of dental alloys and the develop- ment of dental materials: amalgam and gold alloys DA-95	\$6,300	Recommended \$5,000

Special Summer Undergraduate Assistantships

Name Department University		Requested	Approved
Ellis, R.G., Dean, Faculty of Dentistry, Toronto.	Special Summer Under- graduate Assistantships DA-113	\$2,000	\$2,000
Lussier, J-P., Dean, Faculty of Dentistry, Montreal.	Special Summer Under- graduate Assistantships DA-116	\$2,000	\$2,000
MacLean, H.R., Dean, Faculty of Dentistry, Alberta.	Special Summer Under- graduate Assistantships DA-111	\$2,000	\$2,000
McCutcheon, J., Dean, Faculty of Dentistry, McGill.	Special Summer Under- graduate Assistantships DA-115	\$2,000	\$2,000
McLean, J.D., Dean, Faculty of Dentistry, Dalhousie.	Special Summer Under- graduate Assistantships DA-112	\$2,000	\$2,000
Neilson, J.W., Dean, Faculty of Dentistry, Manitoba.	Special Summer Under- graduate Assistantships DA-114	\$2,000	\$2,000

Major Equipment

<u>Name</u> <u>Department</u> <u>University</u>	<u>Equipment</u>	<u>Requested</u>	<u>Approved</u>
Baril, C. Orthodontics Montreal	Grass Model 7-A Polygraph with integrator DE-26	\$4,000	\$2,500
Dowse, C.M., Pritchard, E.T., Kleinberg, I., Hamilton, I. Oral Biology Manitoba	Two scaler/timer assembly for Nuclear Chicago Liquid Scintillation System Vanguard chromatogram autoscanner with glass plate attachment DE-13	\$7,938	\$4,000
Hunt, A.M. Dental Research Toronto	(Beckman) Sharp Lowbeta 11	\$5,746	WITHDRAWN
Nikiforuk, G. Dentistry Toronto	Amino Acid Composition of Enamel Protein DE-27	\$8,202	\$8,200

Special Major Equipment

<u>Name</u> <u>Department</u> <u>University</u>	<u>Equipment</u>	<u>Requested</u>	<u>Approved</u>
Paynter, K.J. Dentistry Toronto	Philips Model EM210 Electron Microscope attachments and accessories DE-28	\$43,000	Recommended

In summary a total of forty-one new applications were considered. Twenty-nine grants amounting to \$158,000 were recommended. Of these, three grants were for major equipment (\$14,700) and two were for new term grants (\$19,500). One grant application and one major equipment application were withdrawn. A further \$12,000 was awarded to six Dental Faculties (\$2,000) for two Special Summer Assistantships in each school. These grants were made to the Deans of each Faculty.

It was MOVED by Dr. R.M. Grainger and SECONDED by Dr. L.E. Francis
THAT grants be made in the amounts specified.

CARRIED

During the discussion of the applications for those grants which were requested by attending members of the Committee, each committee member left the room until his case had been discussed and the recommendation made.

The application of Dr. K.J. Paynter, upon recommendation from the Committee, must be presented to the Special Major Installations Committee for a final recommendation.

The Committee agreed to recommend Dr. Paynter's application on the condition that he supply additional information emphasizing his need for the electron microscope and its proposed use.

The Committee then considered, for recommendation only, one application for a travel grant as follows:

<u>Applicant</u>	<u>Purpose of Travel</u>	<u>Requested</u>
Leung, S.Wah Dentistry Br. Columbia	To participate in the Ciba Foundation Symposium in "Caries-Resistant Teeth", to be held in London, England, June 23-25, 1964, and to visit several English and European Dental Schools.	\$700

The Committee agreed that Dr. Leung's application should be recommended. Payment will be made from grant funds provided for this purpose.

13. Allotment for 1964-65

The Committee allotment for 1964-65 was recommended for allocation as follows:

1 Scholarship	\$ 8,500
2 Associateships	21,800 *
11 Fellowships	45,500
6 Term Grants	45,500
24 Annual Grants	109,800
3 Major Equipment Grants	14,700
Comm. Travel & Admin.	<u>2,500</u>

TOTAL \$248,300

* Plus applicant's share of cost of University pension plan, etc.

14. Forms and Regulations

The Committee considered at length the problem of bringing the literature of the Committee up to date and making any revisions necessary. All informational material was revised by the Committee, in most cases to agree with the recent policies of the Medical Research Council.

The grant application form prepared by the Special Forms Committee was accepted, with minor alterations, by the Committee.

It was agreed to have all forms available both in French and in English.

The Committee decided to retain the Referee Report Form used this year. However, it was proposed that the Executive should study the question of supplying a rating scale on the Referee Form, in order that priorities might be allotted to applications submitted.

It was MOVED by Dr. I. Kleinberg and SECONDED by Dr. R.V. Blackmore
THAT the forms and informational material be accepted as revised by the Committee.

CARRIED

The Committee then discussed at length the booklet, Associate Committee on Dental Research, Extramural Program.

It was MOVED by Dr. L.E. Francis and SECONDED by Dr. R.M. Grainger
THAT this booklet be completely revised and reissued every three years and that any changes during this time be included on separate inserts in the previous issue.

CARRIED

15. Support of Dental Research in Canada

The Chairman presented a report on the support of Dental Research in Canada by agencies other than the National Research Council. These included the Department of National Health and Welfare, the Canadian Dental Association, United States Government agencies, such as the National Institute of Health, and industrial firms, both American and Canadian, such as Lever Brothers, Proctor and Gamble, and Colgate-Palmolive.

Present support of dental research in Canada is as follows:

<u>Source of Support</u>	<u>Amount *</u>
National Research Council	\$167,000
Medical Research Council	8,300
Canadian Dental Association	2,000
Dept. of National Health and Welfare	53,036
Industry (Canadian & American)	51,100
United States Public Health Service	<u>34,250</u>
TOTAL	\$315,686

* The amounts listed are approximations only.

16. Private Donations

It was brought to the attention of the Committee that the Council is able to receive and administer funds donated for research purposes in fields where research is supported by NRC grants.

The Committee discussed the possibility of making this fact known to the public. It was agreed that Dr. R.C. Connor would arrange for publication of an announcement regarding donations of funds to the Associate Committee on Dental Research in the Health and Welfare Journal.

17. Fluoridation

The paper "The Accumulation of Skeletal Fluoride and Its Implications" by J.R. Marier, Dyson Rose, and M. Boulet (Archives of Environmental Health, vol. 6, pp 664-671, May, 1963) had been brought to the attention of the Committee and a request made for a statement regarding the Committee's stand on the subject.

After discussion it was MOVED by Dr. A.M. Hunt and SECONDED by Dr. A.J. Rhodes THAT the Associate Committee on Dental Research as a body was not constituted to express an opinion concerning fluoridation unless specifically requested to do so by the President of the National Research Council and unless asked to make a definite investigation of the subject.

CARRIED

18. Bibliography on Caries Research

The Chairman brought to the attention of the Committee the need for a re-edition of the Bibliography on Caries Research.

It was pointed out that due to the vast amount of material now available, such an enterprise would be beyond the capacity of a single human being, and that expensive mechanical equipment would be needed.

Since agencies in the United States were undertaking such a re-edition, it was agreed that nothing further could be done by the Committee at the present time.

19. Intercommittee Representation

The Chairman drew to the attention of the Committee a recommendation that closer liaison among NRC Grant Selection Committees and Associate Committees be brought about through cross representation.

Following discussion it was MOVED by Dr. A.J. Rhodes and SECONDED by Dr. R.M. Grainger THAT this programme should be initiated by having the Chairman of the Dental Committee attend the meeting of the Grant Selection Committee Conveners at which the allocation of funds is discussed and decided.

CARRIED

20. Membership of Committee

The Chairman informed the Committee that one new member should be elected to replace Dr. R.L. de C.H. Saunders who was retiring. He also informed them that he (Dr. J-P. Lussier) had completed his first three-year term and was eligible for reappointment for a second three-year term.

It was MOVED by Dr. L.F. Belanger and SECONDED by Dr. A.G. Gornall THAT Dr. J-P. Lussier be reappointed for a second three-year term, terminating 31 March 1967.

CARRIED

The following new member was duly nominated and elected: Dr. F.W. Fyfe of Dalhousie University.

It was MOVED by Dr. R.L. de C.H. Saunders and SECONDED by Dr. I. Kleinberg THAT Dr. F.W. Fyfe be appointed for a three-year term terminating 31 March 1967.

CARRIED

It was MOVED by Dr. R.M. Grainger and SECONDED by Dr. W.C. Wilt THAT Dr. J-P. Lussier be reappointed Chairman for a further term of one year, commencing 1 April 1964.

21. Estimates for 1965-66

It was decided that since there appeared to be the likelihood of increasing numbers of applications for research grants and scholarship support, the Committee should submit a request for \$350,000 to carry out its obligations in 1965-66.

22. Fields of Research Supported

The Chairman noted that in the past the Committee had not encouraged applications for grants to support clinical research. After discussion it was agreed that in the future, the fields of research supported by the Committee should be broadened to include clinical research.

It was therefore MOVED by Dr. I. Kleinberg and
SECONDED by Dr. L.E. Francis
THAT the Deans of Canadian Dental Schools be
encouraged to invite their staff to think in
terms of the Associate Committee on Dental
Research supporting clinical research.

CARRIED

23. Publications of Grantees

The Chairman informed the Committee that a survey
had been sent out to Dental grantees of the past five years
requesting a list of publications arising out of research
supported by the Associate Committee; but that response had
been poor.

It was agreed that a follow-up letter should be
sent to those who had not replied requesting immediate
action.

24. Progress Reports

The Chairman drew attention to the greatly increased
bulk of grantees' progress reports and the inconvenience this
entailed.

In view of this, it was MOVED by Dr. A.G. Gornall
and SECONDED by Dr. L.E. Francis
THAT in future, only the summary of each progress
report be included in the Minutes.

CARRIED

25. Conference of Canadian Dental Research Workers

A discussion took place with regard to the plans
for a third Conference on Dental Research, similar to those
held in 1961 and in 1962. It was noted that such a
Conference would require a specific proposal and request for
funds.

It was therefore MOVED by Dr. A.J. Rhodes and
SECONDED by Dr. R.C. Connor
THAT a third Conference of Canadian Dental
Research workers be held, and that a sum of
money be appropriated from the National Research
Council for this purpose.

CARRIED

26. Date of Next Meeting

A discussion took place regarding the possibility
of holding an Executive Meeting in the fall to review the
business of the Committee up to that time.

Grantee

It was MOVED by Dr. L.F. Belanger and SECONDED by Dr. R.V. Blackmore THAT in the interest of greater Committee administrative efficiency, a meeting of the Executive be held in the fall.

CARRIED

The next meeting of all members of the Committee would be held at approximately the same time next year.

The meeting adjourned at 1:15 p.m. on 6 March 1964.

Anderson, J.P.	DA-85	A
Berill, C.		B
Blackmore, R.V.	DA-102	C
Bell, J.P.		D
Donohue, W.B.		E
Dowse, C.M.	DA-81	F
Edmund, A.B.	DA-104	G
Francis, L.	DA-88	H
Jordan, R.E.	DA-106	I
Karloff, Z.	DA-90	J
Karloff, Z.	DA-107	K
Khairat, O.	DA-101	L
Kleinberg, I.	DT-78	M
Lussier, J.P.	DA-70	N
Menz, Z.	DA-72	O
Pritchard, E.T.	DA-109	P
Saunders, E.L. de C.H.	DA-105	Q
Spouge, J.D.	DA-93	R
Tuba, J.	DA-94	S
Woodside, D.B.	DA-110	T
Zoudeleis, W.Y.	DA-95	U

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APPENDIX A

<u>Grantee</u>	<u>Project</u>	<u>Appendix</u>
Anderson, D.L.	DA-86	A
Baril, C.	DA-107	B
Blackmore, R.V.	DA-102	C
Dale, J.G.	DA-99	D
Donohue, W.B.	DA-100	E
Dowse, C.M.	DA-81	F
Edmund, A.G.	DA-104	G
Francis, L.	DA-88	H
Jordan, R.E.	DA-106	I
Kasloff, Z.	DA-90	J
Kasloff, Z.	DA-107	K
Khairat, O.	DA-101	L
Kleinberg, I.	DT-78	M
Lussier, J.P.	DA-70	N
Mezl, Z.	DA-79	O
Pritchard, E.T.	DA-109	P
Saunders, R.L. de C.H.	DA-105	Q
Spouge, J.D.	DA-93	R
Tuba, J.	DA-94	S
Woodside, D.G.	DA-110	T
Youdelis, W.V.	DA-95	U

APPENDIX A

Summary of Progress Report 1963

D. L. Anderson

University of Toronto

DA-86: Histochemical investigation of cell cytoplasm and intercellular interfibrillar material.

In periodontal disease, products of oral microorganisms in crevicular plaque are believed to be responsible for the development and persistence of the lesions. Whether the lesions develop or not, seems to be determined by differences in tissue defensive activity in the periodontium, rather than due to differences in the microorganisms. Periodontal tissue reaction may be inadequate, or it may be excessive in respect to penetration into the alveolar bone and periodontal membrane. In either case, loss of supporting tissue occurs.

Thus it is important to determine factors which increase and decrease tissue reactivity, and also develop a method of objectively testing the state of individual tissue reactivity. It is important to determine if the tissue reactions of people with periodontal disease are different from those without lesions. If they are different, then how can we increase and decrease the reactions to make them more effective and efficient?

Granuloma formation is a connective tissue reaction which can be experimentally evoked and evaluated. Carrageenan and cotton pellet subcutaneous implants are commonly used. Intradermal spread of the dye, Jan blue, during 24 hours after injection is largely dependent upon a local inflammatory reaction, with phagocytosis of the dye. The degree of dye spread indicates connective tissue permeability and reactivity.

A monamine oxidase inhibitor, iproniazid, has been reported to cause proliferation of connective tissue. By inhibiting monamine

idase, serotonin is not destroyed, and the serotonin stimulates the connective tissue. A similar, but stronger monamine oxidase inhibitor, Marplan, was given to rats in their food for 42 days. To some rats large doses of dilantin were given as well. Gingival tissue fibroplasia often occurs in humans taking dilantin. Such tissue contains less serotonin inhibitor. However, neither of these drugs affected the rats' gingivae.

Bone marrow in the maxillae of the animals on Marplan plus dilantin stained more deeply with basic dyes and contained more mast cells than the control diet animals. Cotton pellets soaked in serotonin did not induce a larger granuloma than the control water soaked pellets did, even when the animals were taking the monamine oxidase inhibitor. The pellets from the animals taking Marplan weighed significantly less than the pellets from the animals on the control diet. Wound healing was normal in all groups.

Since it was observed last year that Azure A significantly reduced carrageenan granuloma formation, further experiments with Azure A were instituted. The effect on wound healing was investigated. Azure A was given systemically, topically and by local injection. Azure A was compared to trypan blue in regard to formation of cotton pellet granulomata and intradermal spread. Azure A did not interfere with the healing or reduce the granulomata. It is unlikely though that the Azure A was available to block the sulphate groups at the time of tissue formation, in spite of the various methods of administration tried. It was not possible to place the dye at the right place at the right time. Existing acid mucopolysaccharides in the blood serum and intercellular connective tissue apparently immediately bound the dye. Azure A does not spread intradermally.

the dye itself does not seem toxic, but an injection into the skin results in cell necrosis. Azure A in cotton pellets resulted in significantly heavier granuloma than did trypan blue; and trypan blue saturated pellets resulted in significantly heavier granuloma than did water soaked ones.

It was suggested that the amount of intradermal spread of trypan blue in 24 hours is an indicator of the functional condition of connective tissue. The amount of spread of trypan blue was compared to the weight of granulomata produced by cotton pellets and carrageenan. Individual correlation between spread and granuloma formation was not good, but a trend pointed towards a reciprocal relationship between spread and granuloma weight.

1. When conducting an experiment involving muscle action with the use of the electromyograph, one should use identical electrodes on all muscles which are under observation.
2. The temporalis muscle is subject to a great variability in its shape and size.
3. For the majority of the subjects, the length of the fibers of the temporalis muscle are variable but in direct relation to the position of actual muscular fibers that are found with the electrode.
4. This tendon extends upward and inward, thus increasing the actual muscular tissue of the temporalis muscle.
5. On the part of the temporalis muscle that can be reached with surface electrodes, there are very few strictly vertical and horizontal fibers in relationship to the horizontal Frankfurt plane. The oblique fibers are in majority.
6. The posterior surface electrode is very difficult to place by the usual palpation method. There is a possible risk to fall away from the muscular tissue and there is also a very slight chance of placing the electrode on the actual horizontal fibers.
7. The template used in this study has a definite place in electromyography and is an improvement on our past techniques. The palpation method of electrode placement is subjected to many variations if we compare it to the use of a template for electrode placement.
8. It is possible to make a permanent E.M.G. preparation on a monkey and to study his chewing motions.
9. All elevator muscles studied, i.e., temporalis and masseter participate in the elevation of the mandible.
10. The difference between the deep and superficial fibers of the masseter muscle is minimal.
11. In all sessions studied, the superficial fibers were more active than the deep fibers.
12. On a monkey, an occlusal interference shows its influence on the muscular pattern within ten minutes.

Summary of Progress Report
1963C. BarilUniversity of MontrealDA-107: Electromyography of Vth cranial nerve muscles

Within the past fifteen years, the electromyograph has been introduced into the field of dental research. With the addition to the armamentarium of research equipment, it has become possible to study the electrical impulses of the muscles innervated by the Vth and VIth cranial nerves, and to determine more accurately their individual functions and actions. As in all new fields of scientific investigations, there has been a great variety of methods and procedures used. This has made it extremely difficult to compare the different research activities conducted. This lack of standarization was deplored by Prugansky and Ralston and most workers in the field.

Since two years, an effort was made to correlate different ideas on the subject of electromyography and also attempt to verify whether definite variations could be obtained from the use of multiple technique.

Anatomical and physiological studies on human were carried out. Also an animal study was initiated which proved to be usefull. The following conclusions are to be retained.

1. When conducting an experiment involving muscle action with the use of the electromyogram, one should use identical electrodes on all muscles which are under observation.
2. The temporalis muscle is subject to a great variability in its shape and size.
3. For the majority of our subjects, the lenght of the tendon of the temporalis muscle was variable but in direct relation to the portion of actual muscular fibers that one could reach with the electrode.
4. This tendon extends upward and inward, thus decreasing the actual muscular tissue of the temporalis muscle.
5. On the part of the temporalis muscle that can be reached with surface electrodes, there are very few strictly vertical and horizontal fibers in relationship to the horizontal Frankfort plane. The oblique fibers are in majority.
6. The posterior surface electrode is very difficult to place by the usual palpation method. There is a possible risk to fallaway from the muscular tissue and there is also a very slight chance of placing the electrode on the actual horizontal fibers.
7. The template used in this study has a definite place in electromyography and is an improvement on our past techniques. The palpation method of electrode placement is subjected to many variations if we compare it to the use of a template for electrode placement.
8. It is possible to make a permanent E.M.G. preparation on a monkey and to study his chewing actions.
9. All elevator muscles studied, i.e. temporalis and masseter participate in the elevation of the mandible.
10. The difference between the deep and superficial fibers of the masseter muscle is minimum.
11. In all actions studied, the superficial fibers were more active than the deep fibers.
12. On a monkey, an occlusal interference shows its influence on the muscular pattern within ten minutes.

Associate Committee on Dental ResearchPROGRESS REPORT

December, 1963.

Investigation: Factors influencing the propagation of Herpesvirus hominis in animal cell cultures and in experimental animal infections.

Investigator: R. V. Blackmore.

Supported at the University of Alberta by: N.R.C. Grant #DA-102.

Work has been underway for a six-month period. During that time a tissue culture laboratory has been placed in operation. Stocks of virus sufficient for two years of experimental work have been propagated, titrated and stored. Suspensions of tissue components, to be used as control fluids in subsequent experiments have been prepared, and two assistants have been trained to carry out the routine duties of cell culture as well as herpes-virus and vaccinia virus assay procedures. Animal housing has been arranged and equipment has been installed to care for pregnant rabbits and their litters for a subsequent one year period. The work, to date, has been preparatory to the undertaking of the experimental program and may be summarized as follows:

1. Animal Cell Culture.

A tissue culture incubator (Wedco #2-17, supplied as capital equipment by the University of Alberta), has been installed and put into operation. The controlled carbon dioxide - air - water vapour atmosphere of this incubator permits the culture of animal cells in unstoppered bottles and in Petri dishes.

2. (a) Cell lines.

Cultures of L cells as well as F-L cells have been established and are now available in quantity on a routine basis.

(b) Cell explants.

Two assistants have been trained to make primary cultures from embryonic tissues (see training of assistants, below).

3. Virus Stocks - (Preparation, titration and storage)

1. A reserve stock of Herpesvirus hominis has been propagated by mouse-brain passage, sealed in glass ampoules and stored in a carbon dioxide ice chest.

2. A working stock of Herpesvirus hominis has been propagated in L cell cultures and stored as above.

3. A stock of Vaccinia virus (which will be used as an "indicator" virus in the assay of viral inhibitory factor), has been propagated in L cell cultures.

4. Virus Assay.

1. Embryonated chicken eggs.

A reliable "hatchery strain" of embryonated chicken eggs has been tested and utilized for the assay of the stock virus suspensions. The same strain of 12-day embryonated eggs will be used for Herpesvirus titration in subsequent experimental work.

2. Plaque assay.

A routine plaque assay for Vaccinia virus, as used by the grantee in previous work, has been established in the present laboratory.

5. Herpesvirus Inhibitory Factor.

A stock of control fluids, prepared from uninfected L cells, has been stored for future use.

6. Training of Assistants.

Mr. R. Cheng, a first year dental student, was employed as a Summer Research Assistant during June, July and August, 1963. During this time Mr. Cheng assisted in setting up the laboratory and was trained to assume responsibility for the preparation of media and glassware, as well as for the routine culture of cell lines and explant cultures of embryonic chicken tissue. Also, he prepared the stock of control fluids (mentioned in item 5, above), from L cells which he had propagated.

Miss D. Seifert, a registered medical technician, has completed three months of employment. She has received the same training as that acquired by Mr. Cheng and has proven to be competent and reliable in routine duties.

7. Animals.

Space has been acquired in the Vivarium of the Medical Sciences Building to house rabbits for the ensuing year. Cages suitable for pregnant rabbits and their litters have been purchased and installed. Arrangements have been completed with the Faculty of Medicine, University of Saskatchewan, to provide, as required, pregnant rabbits suitable for the animal experiments which are scheduled to start within the next month.

METHODS:

A. It has been determined by the applicant that a Herpesvirus Inhibitory Factor (H.V.I.F.) is produced in L cell cultures as a response to infection with stock herpesvirus suspensions and assay procedures for this factor have been systematized. A similar H.V.I.F. is produced when L cell cultures are exposed to heat-denatured stock herpesvirus. It has been assumed that H.V.I.F., like influenza interferon, is a cellular product (protein) which is formed by cells in response to a heat-denatured, foreign nucleic acid. Herpesvirus propagation is accompanied by the production of adventitious D.N.A. (10^6 times as much as is incorporated into virus particles). It is of interest, therefore, to determine the relative importance of viral D.N.A. and adventitious D.N.A. in their respective abilities to stimulate the production of H.V.I.F. D.N.A. preparations (both native and denatured) from three sources will be tested:

- (i) Stock herpesvirus suspensions.
- (ii) Virus particles.
- (iii) L cells at varying time periods following herpesvirus infection.

The results of these tests should indicate which D.N.A. source is primarily responsible for H.V.I.F. production.

In addition, once the best stimulus for H.V.I.F. production in L cells has been established, high molecular weight R.N.A. (Messenger R.N.A.) will be extracted from L cells which are yielding H.V.I.F. Such R.N.A., in turn, will be introduced to fresh L cell cultures in order to assess the propensity of messenger R.N.A. to confer upon a new cell population the ability to synthesize H.V.I.F.

B. Herpesvirus has been shown to pass through the placental barrier of rabbits and to enter the foetus where it can be recovered as infectious particles. Those rabbits which are exposed to herpesvirus, in utero, should, theoretically, be immunologically tolerant to herpesvirus as young adults.

It is proposed to inject pregnant rabbits intravenously with high-titer herpesvirus suspensions during the last week of gestation. The young from such females would be used, along with control animals in the following ways:

- (i) Explant cultures of the tissues of embryos and young adults will be examined at suitable intervals of time for the presence of persistent herpesvirus.
- (ii) It is proposed to ascertain whether normal and immunologically tolerant rabbits respond in different ways to herpesvirus infections.
- (iii) It will be determined whether the cells in explant cultures from immunologically tolerant animals are capable of H.V.I.F. production.

SUMMARY OF PROGRESS REPORT

J. G. Dale

University of Toronto

DA-99: The study of bone resorption by implantation and autoradiographic methods

Included in the application for a grant in aid of research, operating grant

DA-99, 1962-63, were five "specific aims" of proposed investigation:

1. to determine whether bone resorption can occur without the presence of osteoclasts and other cells,
2. to study various local conditions under which multinucleated cells appear in the tissues, and to determine whether resorption is taking place,
3. to study the local and systemic influence of hormones and nutrients on bone resorption,
4. to observe the resorptive process in the living animal with and without the presence of cells,
5. to study the ultra structure of resorbing bone and multinucleated cells.

Specific Aims No. 1, 2, and 3 are dependent on the use of three radioactive isotopes: Ca^{45} to represent the inorganic component of bone, S^{35} to represent the ground substance fraction of the organic component, and tritiated glycine to represent the collagen fraction of the organic component.

A series of preliminary experiments were designed to establish specific dosage levels of each isotope for the proposed investigations. The preliminary experiments have been completed. The dosage levels and the method of administration have been established: Ca^{45} , 1 microcurie per gram of body weight administered I.P. in 3 injections, S^{35} , 5 microcuries per gram of body weight administered I.P. in 2 injections, and tritiated glycine, 1.5 microcuries per gram of body weight administered I.P. in 1 injection.

Major experiments utilizing Ca^{45} and S^{35} have been carried out according to the procedure described in the original application. Autoradiograms for the Ca^{45} experiments are being analyzed at present. The S^{35} experiments are being processed histologically and autoradiographically. The tritiated glycine experiments are being conducted at present according to the same procedure.

Preliminary investigations have been conducted to establish standard procedures in relation to Specific Aim No. 3:

- a) a series of rats have been injected with Lilly parathyroid extract to establish the dosage level and the method of administration,
- b) parathyroid gland tissue and scapulae have been removed from a series of rats and placed in double chamber diffusion chambers.

Both series of experiments are being processed histologically.

Much time and effort has been devoted to the design and construction of the time-lapse cinematographical equipment related to Specific Aim No. 4. A Bolex M16 Reflex camera and a Stevens time-lapse apparatus have been purchased and installed on the Zeiss Standard Universal Microscope. An apparatus has been designed and constructed for the purpose of stabilizing an anesthetized animal together with the bone implant in the time-lapse cinematographical equipment. Testing experiments are in progress.

A grant application has been submitted by the Division of Dental Research, Faculty of Dentistry, for funds to purchase a Phillips "200" Electron Microscope. When the microscope becomes available, then work will proceed on Specific Aim #5.

A series of control experiments, utilizing tritium labelled thymidine, and designed to study cellular behaviour associated with bone resorption under the

influence of an applied force, have been conducted.

Having completed the control studies, a series of experiments are being designed and conducted to study cellular activity associated with bone resorption under the influence of an applied force, and various hormonal and nutritional conditions. The first series involves the injection of 6-n-propyl -2-thiouracil to create a hypothyroid condition.

A series of supplementary experiments, designed to study cellular activity in the developing temporomandibular joint, have been conducted utilizing tritium labelled thymidine.

Having completed the control studies, a series of experiments are being designed and conducted to study cellular activity in the developing temporomandibular joint under various hormonal and nutritional conditions. The first series which is completed involves the injection of 6-n-propyl -2-thiouracil to create a hypothyroid condition.

Jack G. Dale
December, 1963

PROGRESS REPORT TO THE ASSOCIATE COMMISSION ON DENTAL RESEARCH

1963

GRANT NO.

C.M. Donohue

University of Montreal

PROJECT NO.

Summary of Progress Report
1963W. B. Donohue:

University of Montreal

DA-100: Glycogen content and distribution in young and aged buccal mucosa

1. Little difference could be detected between the various animal groups using the intensity of the Hotchkiss P.A.S. stain as an index of glycogen concentration. The intensity of the stain varied little even when the quantitative measurements showed a considerable increase in the amount of glycogen present.
2. Quantitative measurements indicate an increase in the glycogen content of the mucosa with age, and with exposure to cold, when the Syrian Golden Hamster is used as the experimental animal.
3. Quantitative measurements showed no variation in the glycogen level of the oral mucosa with age when the white albino rat was used. However, exposure to cold resulted in an increase in the glycogen content of the oral mucosa.
4. In this interim report no conclusions can be arrived at yet regarding the effects of local anesthetics and carcinogens on the glycogen content of the oral mucosa.
5. Parts of the experiment will be repeated during 1964 using larger groups of animals. It is expected that the project will be completed during the coming year. No further funds are requested at this time.

original specific activity.

PROGRESS REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH
1963

GRANTEE: C.M. Dowse University of Manitoba

PROJECT NO. DA-81

PROJECT TITLE: Metabolism of Periosteum

SUMMARY OF REPORT

Continuing work on this project during the past year can be summarized briefly as follows:

1. Increase in medium glucose concentration over the range 0.4 - 1.6 mM produced the following results during the incubation of calvaria in phosphate and THAM buffered media. [Tris(hydroxymethyl)aminomethane].
 - (i) The glucose utilization by the tissue increased uniformly in THAM media while in the phosphate medium there was no increase in utilization beyond a glucose concentration of 1.0 mM (see 2 below).
 - (ii) The production of lactate increased in the THAM media, but was variable and showed no consistent increase in the phosphate medium (see 3 below).
 - (iii) The fraction of the glucose- $\text{U-}^{14}\text{C}$ utilized which appeared in the lactate formed appeared to increase slightly in the THAM media and to decrease in the phosphate medium, with increase in glucose concentration. It was, however, consistently higher in the former medium, about 40%, than in the latter, 20 - 33%.
 - (iv) The specific activity of the lactate produced was quite consistent in both media at different glucose levels, and increased linearly in both cases. At each glucose concentration it was approximately twice as great in the THAM medium as in the phosphate medium relative to the original specific activity.

2. Separation and assay of the glucose- C^{14} remaining after incubation demonstrated that its specific activity had fallen during incubation.

The tissue was therefore releasing glucose of lower specific activity, presumably due to the action of a glucose-6-phosphatase. From the change in glucose specific activity and assuming that the formed glucose was of zero specific activity an approximate minimal value for the quantity of glucose released was calculated. This value was lower in THAM than in phosphate medium and ranged up to 50% of the net glucose utilization determined by chemical assay.

3. The lactate produced was divided on the basis of its specific activity into that fraction which was derived from the glucose- C^{14} and that fraction arising from unlabelled sources.

(i) In both media the % of lactate from glucose- C^{14} was very consistent in duplicate flasks at any given glucose concentration. The variability in net production rate in phosphate medium noted earlier is therefore interpreted as being due to factors influencing glycolysis beyond the point at which endogenous dilution was occurring.

(ii) The production rate of C^{14} -lactate from glucose- C^{14} was seen to increase in both media in a reasonably linear fashion.

(iii) The quantity of lactate produced from unlabelled sources in the THAM medium was almost constant over the range of glucose concentration studied. It was lower than the same fraction produced in the phosphate medium.

4. It was shown in preliminary experiments that $C^{14}O_2$ produced during successive time periods during the incubation of calvaria in medium

containing glucose-U-C¹⁴ could be satisfactorily collected and assayed using paper moistened with NaOH and direct liquid scintillation counting of the dried paper. Less than 3% of the C¹⁴O₂ was present in the tissue indicating that exchange of the metabolic CO₂ with the large mineral CO₃ pool was minor. The C¹⁴ activity in the CO₂ collected in successive time periods increased throughout the incubations, indicating, if uniform metabolic CO₂ production was assumed, that the "CO₂ pool" in the tissue had not reached a constant specific activity in the 2 1/2 hour incubation period.

5. Parathyroid extract, (acetone dried parathyroid powder, Wilson), was partially purified by the phenol extraction procedure of Aurbach (1959) to yield a preparation with an activity of approximately 240 units/mg.

Preliminary work with Sephadex G-50 indicated that the further purification of the hormone to yield preparations of high activity (Rasmussen, 1962) would be relatively straight forward. To enable an approximate assay of the fractions obtained during separation to be most easily carried out the possibility of utilizing smaller numbers of rats than specified in the Munson (1955) assay procedure was investigated. The glyoxal bis[2-hydroxy-anil] method of Goldstein and Stark-Mayer (1958) for calcium was adapted as a direct method requiring only 20 μ l of serum and it was found in routine use that upwards of 150 determinations could be performed in one day. This enabled serial serum calcium determinations to be carried out on individual parathyroidectomized rats. When the time course of the serum calcium response to PTH injection was available it was found that a pair of animals was adequate for an initial approximate assessment of hormone activity.

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Summary of a Report to the Associate Committee on Dental Research

National Research Council of Canada

Dynamics of Tooth Replacement Processes in Modern Reptiles

Project N.R.C. DA 104

By

A. Gordon Edmund
Royal Ontario Museum
University of Toronto

A large number of live lizards and snakes representing several families were obtained from dealers, and in most cases we have succeeded in keeping these alive for many months. Monthly radiographs under anaesthesia have resulted in graphs showing the progress of tooth replacement at each tooth position. These show that tooth replacement in reptiles is a process which apparently is continuous through the life of the animal. Each tooth is replaced many times between the time of hatching and time of death. Replacement occurs in a pattern of waves passing along the tooth row from back to front along alternately numbered tooth series. These waves, in the data analyzed so far, move toward the front at a rate of two to three tooth positions per month. The total life of each tooth was from four to seven months, from its first appearance until its ultimate loss and replacement in turn. This rate may vary considerably from one species to another, but all necessary data are not yet available.

Exceptions to the regular sequence were seen in the teiid lizards Tupinambis and Dracaena, a feature possibly caused by the crowding out of tooth positions during growth.

A further interesting feature is the apparent addition of extra teeth to the tooth row occurring at the posterior of each tooth series. The use of series of specimens of various ages should add to our knowledge of this aspect of the growth of the dentition.

Many lizards and snakes are currently under observation, but the results of their radiographs will not be available for several months. Representatives of other families and animals with different types of dentitions will be studied as finances permit. The use of finer grain film and a beryllium window X-ray machine promises to give more detailed information from the small animals under study.

The results of the project, to date, are quite satisfactory, and if continued to its conclusion, the project should result in a much firmer understanding of the sequence and rate of tooth replacement in reptiles, as well as give an insight into many collateral problems.

The present literature on dental transplantation contains only one

experimental observation on this problem (Gurainik, S. S. and Shulman, I. S.

Russ. Clinic. W. An., p. 493, 1963). The report briefly refers to an ex-

periment on rabbits similar to the above protocol in which the authors

observed accelerated rejection in one animal but were unable to repeat the

finding. They concluded that dental tissues may be similar to cartilage

in that rejection is weak and delayed. Clinically several recent papers

have reported the existence of rejection with dental transplants on the evidence

that teeth grafted to unrelated individuals resorb in the alveolus.

It is our feeling that clinical rejection on the basis of ankylosis or

connective tissue regeneration can not be equated to immunologic incompetence

and this study is intended to support this hypothesis.

Initially we had proposed to use two commercially available, but

unrelated strains of Syrian hamsters and to devote our first efforts to

establishing the median survival time of a primary skin graft between the

ASSOCIATE COMMITTEE ON DENTAL RESEARCH

PROGRESS REPORT N.R.C. DA-88

Dr. Lyman Francis (Grantee)

The following report is submitted as a progress statement of the work supported by N.R.C. DA-88 entitled "Further studies in tooth transplantation"

Phase C: Preliminary studies on the immunologic component of dental grafts .

To determine immunologic phenomena associated with dental grafts, it was proposed that tooth transplants would be exchanged between non-isogenic hamsters followed in four days by test skin grafts. Then by observing the phenomena of primary or accelerated skin graft rejection, conclusions could be drawn regarding the antigenic potential of tooth transplants.

The present literature on dental transplantation contains only one experimental observation on this problem (Guralnick, W.C. and Shulman, L.B. Dent. Clinic N. Am., p. 499, 1962) The report briefly refers to an experiment on rabbits similar to the above protocol in which the authors noted accelerated rejection in one animal but were unable to repeat the finding. They concluded that dental tissues may be similar to cartilage in that rejection is weak and delayed. Clinically several recent papers deny the existance of rejection with dental homografts on the evidence that teeth grafted to unrelated individuals remain firm in the alveolus. It is our feeling that clinical retention on the basis of ankylosis or host tissue regeneration can not be equated to immunologic incompetence and this study is intended to support this hypothesis.

Initially we had proposed to use two commercially available, but undocumented strains of Syrian hamsters and to devote our first efforts to establishing the median survival time of a primary skin graft between the

two strains. This phase was eliminated by the acquisition of breeding stock from two well defined inbred strains of Syrian hamsters, LSH and CB. A breeding colony was established and the rapid gestation of the LSH strain provided sufficient animals for the preliminary study late this summer.

Prior to the first dental study, multiple test skin grafts were undertaken to become familiar with the techniques of grafting. In this respect, time was spent in the transplantation laboratories at the Peter Bent Brigham Hospital and the Wistar Institute observing their various procedures. Billingham's pinch graft technique was modified to achieve the uniform thickness required for the microscopic evaluation of rejection used at Harvard. The pinch grafts obtained by excising down to the muscle layer and dissecting away unwanted fat and fascia were substituted for grafts in which a plane of cleavage established in the dermal connective tissue was dissected over a previously outline 1 c.m. square of dorsal skin. This yielded a uniform graft which was easily reproducible in each animal.

Procedure: The first dental study was undertaken with 18 LSH and six CB hamsters. The LSH animals were divided into three groups of six. Group "A", the controls, were given intraperitoneal injections of 1 ml. of Eagle's B.S.S. followed in four days by two test skin grafts, an autograft and a CB homograft. Group "B" each received two fragmental CB hamster pulps intraperitoneally in Eagle's B.S.S. followed in four days by two test grafts. Group "C" each received four developing third molars in 1 ml. of Eagle's B.S.S. intraperitoneally followed by the test grafts in four days. The sex of the animals were matched such that female CB tissue was only donated to female LSH animals. The same consideration was given to

male tissues in order to eliminate any sex linked transplant antigens.

The animals were placed in separate cages, they were given food and water as tolerated and the bandages were removed four days after skin grafting.

Results: The evaluations are detailed in Table I. The figures represent the percentage of skin survival as observed grossly. It is the method used by Billingham and others to evaluate rejection phenomena, but will be replaced by microscopic interpretation when differences between the antigenic potential of various dental tissues is to be studied.

Discussion: From the detailed results, several interesting observations can be drawn. The most obvious is the total absence of accelerated rejection in the animals receiving dental tissues. In fact it appears that control animals actually rejected more rapidly than those supposedly sensitized with pulp fragments and whole teeth. There are several possible explanations why dental tissues failed to sensitize the recipient other than the lack of competent transplant antigens. First, the time interval of four days between the insertion of dental grafts and the test skin may have been too brief to allow sensitization to occur or to reach detectable levels. Secondly, the possibility exists that the introduction of tissue into the peritoneal cavity may not be as antigenic as the subcutaneous or intravenous route. Related studies with other tissues suggest this is unlikely. Thirdly, the dose of tissue may have been insufficient to give a response. Antigen-antibody reactions were early shown to conform in part to a dose response curve. The antithesis of this postulate is that the dosage was too large and the immune response was paralyzed. This rather theoretical explanation would account for the apparent delayed rejection in the experimental animals compared with the controls. Paralysis of immune

mechanisms can certainly be achieved with massive amounts of antigen, but it is quite unlikely with the volume of tissue used in this study. The more logical explanation for the delayed rejection seen especially in group "B" relates to the hazard of grafting tissue in areas of rapid hair growth. The pelt on the dorsal surface of the hamster has variable areas of accelerated hair growth which can be easily distinguished by the deeper pigmentation. Unfortunately, some animals have only this type of hair growth and our limited supply necessitated their use. Subsequent control studies confirm that rejection at these sites is erratic and difficult to interpret. In this light we feel that there is probably little difference between the rejection pattern of the controls and experimentals and that they all represent primary rejection. The only definite conclusion that can be drawn from this preliminary study is that within the limits of the protocol, there was no demonstratable sensitization with dental tissues.

The next dilemma is the extremely rapid primary rejection response of seven days. The figure reported in the literature by Billingham for this particular strain combination, CB to LSH, is 10.3 days. It is interesting that the seven day response is not in line with reported primary skin graft rejection in any other specie. We are presently reconfirming this figure in a much larger series of animals and we now have an additional twelve seven-day rejections. The explanation for this finding is not readily apparent. Our technique is markedly different from the method used by Billingham in establishing the M.S.T. of ten days. We feel that our method allows more rapid healing, perhaps earlier vascularization and theoretically faster rejection. This convenient

explanation, however, is not substantiated by others using a similar grafting technique in the mouse where rejection is again in the ten to thirteen range. As stated, we are still gathering data to support this new median survival time before consulting with Billingham on the possible significance.

Phase B. Continued studies on the development of media for in vitro cultivation of teeth.

The inability to satisfactorily store whole developing third molars in tissue culture led to the studies using free pulp tissue obtained from hamster molars under the dissecting microscope. Control sections of pulp recovered in this manner revealed normal intact pulp tissue surrounded by a partially interrupted rim of odontoblast. The fragments, obtained under sterile conditions, were then introduced into various culture protocols.

Procedure: The studies over the past eight months have been designed to either grow or maintain the pulp fragments in a basic tissue culture medium. The primary stock medium consisted of Eagle's balanced salt solution containing 5% chicken serum, penicillin and streptomycin. The pulp fragments were cultured in either of the two routines: (1) Yerganian tubes - a shortened variety of the Leighton tube in which the tissue was planted on a cover glass, allowed to dry for several minutes and then was covered with stock medium (2) Hanging drop cultures in which fragments were imbed in a matrix containing one drop of chicken plasma and one drop of chick embryo extract diluted 20/1 with Eagle's stock medium. The tissues were cultured at 37 C for varying periods of twelve hours to ten days. Both whole pulp tissue and pulp fragments were in-

cluded to distinguish the effect of volume as it relates to diffusion.

Early studies with the culture flasks were complicated by an inability to prevent a rise in the pH to eight or greater. Repeated attempts to relate the alkalinity to contaminated glassware failed and it finally became necessary to charge the tubes with 4% CO₂ to achieve equilibrium. Infection was never a problem. Evaluation of the hanging drop cultures were made daily for microscopic outgrowth and finally by routine H. and E. histology. In later studies, photomicrographs were taken to confirm outgrowth.

Results and Discussion: The following findings relate to a series of experiments conducted under this phase. Yerganian flask cultures in which pH changes were controlled contained viable pulp tissue after 96 hours. Larger fragments, especially whole pulp tissue, began to show central degeneration at this time. Odontoblasts were maintained in the early recoveries, i.e. 96 hours or less, but showed granularity and nuclear changes in the later specimen. Tissue incubated longer than 96 hours regardless of size exhibited progressive cellular degeneration.

Hanging drop cultures were undertaken to assess viability as determined by cellular outgrowth. Visual microscopic evaluations were suggestive but detailed day to day comparisons were difficult since the growth was never abundant. In later studies, using photomicrographs, definite outgrowth in these cultures was demonstrated. As expected, the hanging drop cultures were more sensitive to prolonged storage than those in Yerganian tubes. Tissue would not survive beyond 48 hours in the medium described. Recoveries prior to 48 hours showed good cellular preservation of both odontoblast and pulp element indistinguishable from

control nonstored tissue. The size of the fragment in this technique was not a limiting factor as was suggested in the flask studies, but specimens were generally smaller.

We conclude that lack of diffusion is still one of the chief factors limiting in vitro storage of whole hamster molar pulp. Additional efforts are being made to improve nutrient and diffusion potential of the medium. Renewal of the stock solution in the culture flasks and explanation of hanging drop tissue has been shown to increase the duration of storage but has little practical value in the ultimate goal - a tooth bank. Perfusion methods are being considered as a possible answer to the nutritional problem in the deeper portions of the pulp. An experimental model using a larger tooth than the hamster is being investigated.

Phase A. Continuous studies using the millipore diffusion chamber.

Progress in this area of the study has not been significant; first, because of our inability to develop a satisfactory technique of chamber construction, and secondly, the utilization of most of our available time on phases B and C. The purpose of this phase, the development of an extraoral growth site to evaluate whole teeth stored in phase B, hinges on our ability to store dental tissues in a state where viability must be evaluated by growth potential. Presently we are unable to maintain whole teeth in vitro free of pathological changes which can easily be detected by routine histology, thus development of this phase has not proven urgent.

TABLE I

DAY 7

<u>GROUP A</u>			<u>GROUP B</u>			<u>GROUP C</u>		
Animal No.	Autografts	Homograft	Animal No.	Autograft	Homograft	Animal No.	Autograft	Homograft
26	R(lost)*	R**	30	80	40	36	80	R
27	80***	R	31	80	60	37	70	50
28	80	30	32	80	20	38	80	50
29	90	R	33	90	50	39	90	R
34	100	R	42	R(lost)	R	40	20	R
35	85	R	43	80	30	41	R(lost)	R

DAY 10

<u>GROUP A</u>			<u>GROUP B</u>			<u>GROUP C</u>		
26	R(lost)	R	30	100	20	36	100	R
27	100	R	31	80	70	37	100	40
28	90	R	32	100	R	38	90	20
29	90	R	33	100	20	39	100	R
34	100	R	42	R(lost)	R	40	20	R
35	100	R	43	100	R	41	R(lost)	R

DAY 13

<u>GROUP A</u>			<u>GROUP B</u>			<u>GROUP C</u>		
26	R(lost)	R	30	100	R	36	100	R
27	100	R	31	100	50	37	100	30
28	100	R	32	100	R	38	100	20
29	100	R	33	100	R	39	100	R
34	100	R	42	R(lost)	R	40	20	R
35	100	R	43	100	R	41	R(lost)	R

* R(lost) represents failure to heal

** R represents graft rejection

*** 80 represents percentage of skin surviving by gross examination.

PROJECT DA-106 GRANTEE: R.E. JordanPROGRESS REPORT

Title -- PRIMARY MOLAR DEVELOPMENT IN THE HUMAN FETUS

Methodology-- Approximately fifty human fetuses ranging in crown-rump length from 28 to 200 mm (8 to 21 weeks) have been received from various hospitals in the Maritime area. In addition, ten "term" fetuses have been studied, as well as four infants within the first two years of life. The dental follicles of all specimens were extracted, stained with Alizarin Red S, and examined under low magnification (15 to 50x) using a cycloptic stereoscopic microscope.

Twelve Rh monkey fetuses were also studied in the same manner (at the University of Washington) and their dental morphogenetic stages compared to those of the human sample. In addition, access was gained to a sample of 75 circumnatal fetuses from a separate research project at the University of Washington.

Results--The development of primary and first permanent molar embryonic crowns involves two broad phases a) The precalcification phase, which is characterized by the orderly appearance of the soft tissue "cusps" on the embryonic crown germs. The various stages in the precalcification phase of each molar are distinctive for each tooth. A very definite, characteristic pattern of cuspal differentiation is noted during this phase; b) The calcification phase, in which the various centres of calcification appear at the apices of the cusps in a seemingly rigid sequence. The appearance of the various centres of calcification is followed by an equally rigid, distinctive pattern of calcification, whereby calcified tissue spreads in a regular predictable manner to "join" the separate centres of calcification.

Detailed descriptions of the several stages comprising the precalcification and calcification phases of Molar Ontogeny are now being prepared for publication in book form. (Tentative title- "Prenatal Morphogenesis of the Human Dentition"). The manuscript is nearly complete and will be submitted to the publisher early in the New Year. (Authors- Bertram S. Kraus, F.H.D.
Ronald E. Jordan, D.D.S., M.S.)

The results of the Rh monkey morphogenetic study (as compared to Human molar development) will also appear in the same publication.

The immediate aim of the present research project will be directed towards a more lucid definition of the status of the Human Dentition at birth. Textbooks of Dental Anatomy, Embryology (Wheeler; Noyes; Kronfeld; etc.) are in general agreement that "at birth" the calcified cusp tips of the second primary molars "are still isolated", and, with respect to the first permanent molars,

"sometimes a trace" of calcification is present (Logan & Kronfield). Our studies, on the other hand, indicate that the second primary molars of the "term" fetus show a considerable degree of calcified inter-cuspal coal esence, (usually involving three of the four upper molar cusps and four of the five lower molar cusps); also the first permanent molars, at birth, show at least one and often two, or three centres of calcification present. The apparent disparity between previously published data, and those of the present study, might possibly be due to the fact that the former base their conclusions primarily on Roentgenographic evidence. The present study, on the other hand, using Alizarin Red S staining techniques, detects even minute amounts of calcification, which might conceivably be overlooked using Roentgenographic techniques.

A further aim of the present project is to determine the time and manner of formation of occlusal surface enamel defects (pits, fissures) on primary molar crowns. Examination of the primary molar crowns of over 80 term fetuses indicates that such defects are a post-natal phenomenon, since pits and fissures are not demonstrable during fetal development. There is some indication that the time of calcification (of defect areas) may be of significance, since the second primary molars of many of our term fetuses show that a) the central and distal pit regions are the last areas on the occlusal surface to undergo calcification, and b) calcification of these commonly faulted areas occurs post-natally.

The manner of formation of enamel defects poses an interesting problem. Many anatomists (Black; Wheeler) point out that pits and fissures result from the "imperfect" fusion of the separate centres of calcification (growth centres). Our sample indicates that by the time of birth, calcified fusion between the buccal and lingual cusps of the first primary molars is complete, but as stated previously, evidence of faulty fusion (i.e. fissures, pits) was not encountered in any of the specimens examined.

Kronfield (1935), and Massler and Schour (1940) offer the most acceptable explanation for the mode of formation of enamel defects. They point out that pits and fissures result in the area between two "steep" cusps when the inner enamel epithelium in the immediate region of the intervening groove is trapped by the ameloblasts on the adjacent cusp slopes growing toward each other. This theory, (which to our knowledge is not supported by histological evidence) seems reasonable when applied to occlusal surface defects, but does not account for enamel defects commonly found on the buccal and lingual surfaces of molars and the lingual surfaces of upper incisors. Further research in this area of Dental Morphogenesis might, therefor, be indicated.

Some control studies are now in progress to verify the accuracy of the procedure, before final assessment is made.

A complete and detailed report will follow in the next two or three months, terminating this study.

Respectfully submitted

THE RELATIONSHIP OF DEFECTS IN TEETH
ASSOCIATED WITH INDUCED STRESSES TO THE
HISTOLOGICAL PATTERN OF TOOTH STRUCTURE

PROGRESS REPORT

Project: DA-90

Grantee: Zack Kasloff, Assoc. Prof. Dept. of Dental Materials and
Section of Fixed Partial Denture Prosthesis

Institution: Faculty of Dentistry, University of Manitoba

The final phase of investigation for this study is now nearing completion. It is designed to determine the relationship of cracks induced by various rotary cutting instruments to the component hard structures of teeth.

Two hundred and ten extracted human teeth, in groups of five were used for each of the conditions under investigation. Labial or buccal and proximal surfaces of anterior teeth, bicuspid and molars were included in the study. Only one surface was studied on any given tooth. The technique of fluorescent dye penetration and ultra-violet light inspection was utilized to reveal the defects and follow their course.

Prior to cavity preparation, the teeth were subjected to dye penetration and were photographed. Subsequently, the teeth were prepared with conventional speed, high-speed and ultra-high speed rotary cutting instruments with steel, carbide and diamond cutting tips. Further dye penetration and photography revealed induced cracks.

Each tooth was then partially embedded in a rigid plastic base. The prepared surface of the tooth was left exposed sufficiently, so that grinding of the surface involved could be carried out without interference from the embedding compound. A mounting frame with a positive lock was designed so that it could carry the specimen to an exact position on a hard tissue cutting machine. Increments ranging from .004 inches to .001 inches were progressively removed with a rotating grinding wheel on the machine under a constant water spray. After each increment of removal, the specimen was viewed under the microscope for the presence or absence of a given crack under observation and the surface was photographed. This procedure was repeated for each increment of removal until the crack was no longer visible. The total of increments removed by grinding represented the depth of a crack.

Some control studies are now in progress to verify the accuracy of the procedure, before final assessment is made.

A complete and detailed report will follow in the next two or three months, terminating this study.

Respectfully submitted

Z. Kasloff

The addition of a 25 mm luminar 1:3.5 objective overcame this problem to some extent. Studying the fractured ends enabled us to appraise the quality of the prefabricated cast structures as well as the soldered joint.

Fractured parts were placed together so that the planes of the fractured ends opposed each other. These were embedded in a thermoplastic molding compound by conventional metallurgical mounting techniques and the specimens then polished in preparation for etching and metallurgical evaluation. Various etching techniques commonly employed were tried and modified. The technique selected for application to our studies consisted of using concentrated aqua regia initially, followed by an electrolytic action involving brief contact with a 5% solution of potassium cyanide. This proved to be the best method for our purposes. Kodak Pantomic-X black and white 35 mm. film recorded the grain structures.

Various objectives with different magnification powers were employed. It became apparent that our rather rudimentary type of microscopic equipment was inadequate for proper metallurgical interpretations.

With these problems under control, we are now prepared to carry out intensive studies as proposed.

Respectfully submitted

METALLOGRAPHIC STUDIES OF SOLDERED
JOINTS AND CAST GOLD PARENT METALSPROGRESS REPORT

Project: DA-107

Grantee: Zack Kasloff, Assoc. Prof. Dept. of Dental Materials and
Section of Fixed Partial Denture Prosthesis

Institution: Faculty of Dentistry, University of Manitoba

In compliance with the recommendation of the Associate Committee on Dental Research, research in the above-mentioned area was initiated. It must be mentioned that the amount of the grant awarded was of an order that necessarily limited the scope of investigation. It did enable us to conduct pilot studies for establishing proper techniques for the proposed investigations.

The cast gold parent metal specimens were cast in the form of pins approximately 1.5 inches long x 0.090 inches in diameter. A steel V-block was modified and fitted with clamps to receive a pin previously cut in half with a coping saw. A definite gap distance was provided by means of a feeler gauge inserted between the two parts prior to clamping. Hard sticky wax was used to unite the two portions and the specimen was then embedded in soldering investment. When the set was complete, the wax was flushed out and the gap soldered with one of three fusing range solders.

Specimens were then removed from the investment compound, cleaned of all debris and machined on a lathe so that approximately 0.5 inch of the specimen in the soldered area had a uniform diameter of 0.070 inches. The specimen now had somewhat of a dumbbell shape to it. The enlarged ends were held in specially fabricated self-aligning grips developed specifically for this size specimen. Physical testing in the form of an ultimate tensile test was utilized to determine the strength values and location of fracture. A specific rate of loading was used. This was in the order of .022 inches per minute. Of interest here is that nowhere in the literature is reference made to any rate of loading when similar tests were made. It was found that different rates of loading produced different values for tensile strength before fracture. The fractured ends of specimens were observed and photographed microscopically. Available equipment proved inadequate for obtaining sufficient depth-of-field focus.

SUMMARY OF THE PROGRESS REPORT

Grant No. DA-101

Name : Omar Khairat, M.D., Ph.D., D.T.M. & H., F.R.P.S.
 Department : Prof. Wilt's Department of Bacteriology & Immunology,
 Faculties of Medicine & Dentistry, Univ. of Manitoba.
 Institution : Faculty of Dentistry, University of Manitoba.
 Title of Project : Post-extraction bacteremia, with special emphasis on
 the anaerobes and the antibiotic active against all
 the aerobes and all the anaerobes isolated from the
 circulating blood.

- - - - -

The first part of this project was to take 100 blood cultures from 100 patients, under no antibiotic cover, before and 1 minute after extraction. The second part was to test the organisms isolated, in vitro, against 22 antibiotics. The third part was to try the antibiotic chosen, as an antibiotic cover, for a 100 patients during teeth extraction, in an attempt to solve the problem of preventing subacute bacterial endocarditis resulting from dental extraction.

Among the 64 persons who developed a bacteremia in the first 100 blood cultures were 26 males and 38 females. Race: 32 Canadians of British or French extraction, 8 Red Indians, 11 Ukrainians, 3 Dutch, 3 Poles, 2 Germans, 2 Hungarians, 2 Chinese and 1 Norwegian.

Twenty three had single extractions and 41, multiple. No particular quadrant gave more positive blood cultures than the three others. The question of "rocking" (much trauma) was not a factor either, for 16 had much rocking and 48 (three times as many) had an easy extraction with hardly any rocking. The condition of the gums did not seem to influence the bacteremia, for 18 (27%) suffered from severe periodontitis, 29 (45%) had a moderate degree and 17 (28%) had no noticeable gum disease. The reason for extraction was: caries in 38 patients, periodontitis in 11, both conditions in 7, periapical abscess in 3, root in 3 and wisdom tooth in two. The 1st blood culture in this non-medicated group was taken on February 23, 1962 and the hundredth on November 14, 1962.

The strains isolated were 64 aerobes and 81 anaerobes. The aerobes were viridans streptococci, a Strep. pyogenes Group F, corynebacteria, Neisseria, Staph. aureus, Nocardia and Aerobacter aerogenes. The anaerobes included anaerobic streptococci, Fusiformis, Bacteroides melaninogenicus, other bacteroides, Viellonella orbiculus, and anaerobic corynebacteria. There is no question of contamination in these blood cultures, for none of the 100 samples taken from the circulating blood of the same patients before extraction - by the same technique - yielded any organisms, aerobes or anaerobes.

Although some published papers reported a few anaerobes, the present work records the highest number of anaerobes ever isolated in a series of 64 positive blood cultures following teeth extraction, and the only work where 58 of the strains isolated from the blood were tested against 22 antibiotics in search of one that inhibited, in the lowest concentration used, all the aerobes and all the anaerobes responsible for dental post-extraction bacteremia.

Four such antibiotics were found to fulfil this dream almost completely. The applicant planned to try one of them on a 100 patients as an antibiotic cover before extractions. It was given first on April 8, 1963 in a dose of 250 mg 1 1/2 hours before extraction. The first seven patients yielded sterile blood cultures (a chance distribution), for 6 of the 9 patients that followed, gave blood cultures positive for anaerobic streptococci, viridans streptococci, N. pharyngis, corynebacteria and Bacteroides melaninogenicus. So the dose was increased to 500 mg. Three of 9 patients yielded positive blood cultures. The same dose was tried again but given 2 instead of 1 1/2 hours before the extractions. The two patients tried gave positive blood cultures, one a Viellonella and the other a Fusiformis.

Again the same dose (500 mg) was tried but given 4 hours prior

to the extractions. Two of 6 more patients gave positive blood cultures.

So the dose was finally raised to one gramme given 4 hours before the extraction -and on an empty stomach- to 9 more patients, but even then 3 developed a bacteremia. The dose could not be increased any more for fear of toxicity and it was obvious that that antibiotic proved a failure in sterilizing the blood after dental extraction, for 16 patients in a group of 42 yielded positive blood cultures (38%), a reduction from 64 to 38% which was statistically worthless. The organisms isolated were tested for sensitivity to that antibiotic and they all proved inhibited, and the serum (part of the original blood sample) was assayed for antibiotic content.

A second antibiotic (one of the remaining 3 in vitro-successful ones) was first tried on July 10, 1963 and is still being tried and seems to be going to work, for it has so far been given to a good (though not a large) number of patients and only one positive blood culture has been encountered : a viridans streptococcus.

The applicant is hoping to get a startling result with this antibiotic, better than any so far published, considering the number of cases investigated and the large number of aerobes and anaerobes isolated in the non-protected control group. He is going to take more blood cultures in this series. Also he plans to classify the (1) anaerobic streptococci isolated from the blood, (2) the corynebacteria, (3) the Viellonella and (4) the viridans streptococci. These projects will take years and years to do. He started a piece of work on the nutritional requirements of the bacteremia anaerobic streptococci, as this important group of oral bacteria had little work done on it.

The applicant is sorry not to have given in this progress report, details about the strains isolated, for this awaits full identification, and is sorry not to have named the antibiotics he has been using, for this work has not been published yet.

During this last year four papers were accomplished while the work on this project was going on :

- 1) "A simple spacer for Petri dishes". published in the (British) Journal of Applied Bacteriology (1963) 26 : 232-233.
- 2) "Modernizing Brewer and other anaerobic jars already in use". Accepted for publication in the Journal of Bacteriology (1964).
- 3) "A tube and an autoclavable medium for the fermentation reactions of fastidious anaerobes". Under publication.
- 4) "Routine incubation in a large (80-plate) CO₂ jar". Under publication.

Seven references were mentioned in the detailed Progress Report.

APPENDIX M

PROGRESS REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH 1961 - 1964

GRANTEE: I. Kleinberg University of Manitoba
PROJECT NO. DT-78
PROJECT TITLES: 1. Further Studies on the Role of the Dental Plaque
in the Caries Process.
2. Further Studies on the Effect of Substances Removed
During the Refining of Carbohydrates on Calcium
Phosphate Solubility.

Project 1. Further Studies on the Role of the Dental Plaque in the
Caries Process.

Introduction

Studies on the metabolism of the oral microorganisms in dental plaque, the bacterial films located on tooth surfaces, have been continued. This has been done by observing their behaviour in plaque in situ and in saliva-substrate mixtures in vitro. For the latter system, the microorganisms have been "harvested" by allowing plaque to accumulate on the surfaces of the teeth and then dislodging them by chewing paraffin. The pH changes in the in vitro system under a variety of conditions has been shown to change in much the same way as plaque in vivo, if its cell concentration is increased at least ten-fold after harvesting. Two manuscripts entitled "The Effect of Salivary Sediment Concentration on the Shape of Glucose Concentration-pH Curves in Incubated Saliva-Glucose Mixtures" and "Further Studies on the Characteristics of pH Curves in Incubated Saliva-Glucose Mixtures" establishing this point have now been submitted for publication and copies are attached to this report as Appendices "A" and "B". The main advantages of examining the two systems

simultaneously is that the behaviour of the oral microorganisms in the two can be related, making it possible for the techniques and phenomena more readily developed and understood in the one to be more effectively applied to the study and understanding of the other. The effects of the metabolism of the flora as a whole on the solubility of calcium phosphate and of the salivary mucoproteins is also being studied because of the importance of the former in the caries and periodontal disease processes and the latter in the mechanism of plaque matrix formation.

The in vivo aspects covered by this report were (1) an investigation of the pH levels in gingival crevices located in different regions of the oral cavity (ii) the development of several ultra-micro procedures to be used in future experiments on plaque metabolism in situ and (iii) the development of a suitable procedure for the estimation of calcium that could be adapted to the ultra-micro level.

The in vitro studies that were carried out were (i) an investigation on the factors responsible for the accumulation of carbohydrate by the oral microorganisms from glucose and lactic acid substrates and in turn the effect of this accumulation on the shape of pH curves in the saliva-substrate system (ii) a study to determine whether fluoride affects this accumulation process and these pH curves and (iii) studies on the effect of changes in pH in this system on the solubility of the calcium, phosphate and protein that it contains.

Project 2. Effect of Substances Removed During Carbohydrate Refinement on Calcium Phosphate Solubility.

Introduction

Osborn, Noriskin and Staz (J. dent. Res. 16, 165, 1937 et seq.) showed in in vitro experiments with saliva-carbohydrate mixtures that wholemeal flour contained some factor not contained in refined flour that protected calcium phosphate salts (or tooth hard tissues) from acid decalcification. These experiments were confirmed and extended by Jenkins, Forster, Speirs and Kleinberg (Brit. dent. J. 106, 195, 1959). In this latter study, the factor identified as responsible for this protective effect of wholemeal flour was the phytate molecule. Because the oral microorganisms can produce similar quantities of acid from refined or wholemeal flour, it was concluded that phytates (present in the latter and not in the former) could only have their effect by affecting calcium phosphate solubility in this system. These findings in conjunction with the epidemiological studies of Staz suggested that refinement of carbohydrate is responsible for an increased caries incidence in a population and that the removal of phytates may be a contributing factor towards this increase. During the period that this report covers, McClure showed that caries incidence in rats could be reduced ~~from~~ 77 - 100% when phytate was present in the diet (J. dent. Res. 42, 693-699, 1963). Whether this is a local effect (i.e. in the oral cavity) or a systemic effect is yet to be determined.

Phytates appear to be important in other phenomena as well as in dental caries. For example, McCance and Widdowson (J. Physiol. 101, 42, 1942) and later Krebs and Mellanby (Biochem. J. 37, 466-468, 1943)

carried out experiments showing that with phytate in the diet, the human organism goes into negative calcium balance. This they suggested could cause rickets in low calcium diets.

As a result of their experiments, British flours as a matter of national policy have had calcium carbonate added since 1943 for the purpose of supplementing the low calcium content of the average British diet and to "protect" against the effect of any phytates present in the flour. Walker, Fox and Irving (Biochem. J. 42, 452, 1948) however, showed in experiments carried out for a longer period than those above, that the negative calcium balance observed is only temporary and concluded that the adverse effect on calcium balance of phytates, is questionable. To date no explanation has been provided for these observations with phytates, which would of course have to be provided if they should be shown to be essential in human diets to prevent caries.

quantities An important point that apparently has been overlooked in the results of McCance and Widdowson is that with decreased retention of calcium by the body with phytates present, there is an increased uptake of phosphate. This could mean that the presence of phytates in the gut alters the ratio of Ca to P absorbed, so that the former is reduced while the latter is increased. Because inorganic phosphate levels play an important part in the regulation of intracellular processes in general, this effect might as a result have important implications nutritionally and more particularly may be a factor in any systemic effect that phytates might have on caries. Related to this is the possibility that phytates (or related substances - see below) may be important in the formation of

hydroxyapatite in mitochondrial membranes (Rossi and Lehinger, Biochem. Zeitschrift, 338, 698-713, 1963) and in the transport of calcium and phosphate across cell membranes in general.

Recently phytates have been shown by Morton and Raison (Nature, 200, 429-433, 1963) to function as a "buffer" of the ATP-ADP system in wheat endosperm cells. Their results indicated that the phytate molecule provided a reserve of reactive phosphoryl groups which could function to keep cellular ATP at a maximum level and therefore the numerous synthetic functions of the cell which are dependent upon the level of this compound, also at a maximum level. Some mammalian cells, such as the red blood cell, have been shown to contain phytate; its counterpart, the phospho-inositides seem to be more common, in that they are a constituent of the phospholipides contained in the membrane material of most, if not all, cells. Polyphosphates, recently shown to exist and accumulate in large quantities within certain bacteria (Harold, J. Bact. 86, 885-886, 1963), may also be a related compound and serve similar functions.

As a result of the above and numerous related studies, the present investigator has continued his studies on the effect of phytates on calcium phosphate solubility mainly because once this is answered the investigation of the effect of phytates (or related compounds) on calcium phosphate solubility in plaque, in the lumen of the gut and in cell membranes in general, should be easier. The grantee is of the opinion that answering any one of these will have considerable biological implications.

To study the effects of phytates on calcium phosphate solubility, it was necessary to go back a step and determine whether the hypothesis

proposed by Levinskas and Neuman (J. Phys. Chem. 59, 164, 1953) that hydroxyapatite had no Ksp had any validity. This conclusion of theirs was based mainly upon the observation that changing the ratio of hydroxyapatite to aqueous medium, showed different solubilities of this salt. They suggested the theory of Knapp (i.e. that different particle sizes give different solubilities; Trans. Faraday Soc. 17, 457, 1922) as explanation. For several reasons, this hypothesis was criticized by LaMer (J. Phys. Chem. 66, 973-978, 1962) who provided an alternate suggestion, which the experiments below substantiate. It is fortunate that the theory of Levinskas and Neuman is not correct, because if it were, a study of the effects of phytates on calcium phosphate solubility would have been a much more difficult task than it appears to be.

APPENDIX N

Summary of Progress Report 1963

Grant # D.A. 70 - 1963.

The origin and fate of citrate in bone under various influences.

J.P. Lussier, Faculty of Dentistry, University of Montreal, Montreal, P.Q.

The partially hepatectomized bilaterally nephrectomized (HN) rat was found to be a valuable biological preparation to study tissues which could be affected by hypercitricemia as well as influences which may bring changes in the rate of production on utilization of citrate. More details were required to establish as to how this preparation could best serve. It was found that the extent of partial hepatectomy is important. The removal of two lobes have twice as much influence as one lobe. It was also found that it took about eight hours after nephrectomy before a plateau is reached in blood citrate. Several factors were investigated in order to find out how their influence could compare in the HN and in the normal animals. Insulin, parathyroid extracts and sodium fluoroacetate were tried. The HN animal reacted similarly as the normal. Due to hypercitricemia the differences were much more obvious in the HN animals.

The teeth of the rats submitted to hepatectomy, nephrectomy and both hepatectomy and nephrectomy were analyses for their content in carbon dioxide and citrate. It was found that their carbon dioxide content is not affected by hypercitricemia. The citrate content on the contrary is highly influenced by the blood citrate level in the dental structures which are undergoing mineralisation, namely in the

E. Mail

University of Montreal

Summary of Progress Report - 1963

DA-79: Experimental modifications of the permeability of the superficial zone of enamel caries

apical portion of the growing incisor. This finding may help to a better understanding of the relationships between citrate and other factors upon which lie the resistance of the dental tissues.

A ground surface is prepared longitudinally through the tooth and through the center of the lesion. This surface is accurately covered with a transparent varnish, resistant and impermeable to the solutions used. Through the varnish, the degree of penetration of the stain entering through the natural surface of the lesion can be observed with a stereoscopic microscope.

Progress of the penetration of toluidine blue in initial carious lesions was measured.

The permeability of initial carious lesions was compared before and after treatment with the following solutions:

the retarding solution of McQuarrie, Gusto and Piggan

saturated solutions of calcium phosphate in

lactic acid

saturated solutions of dental tissue in lactic acid

lactate buffer solution.

Solutions of calcium phosphate and dental tissue can also slow the penetration of staining solutions in initial carious lesions. Certain lesions easily permeable at the beginning have been rendered impermeable.

Lactate buffer solution at a pH of 5.35 can penetrate in lesions which are relatively impermeable for aqueous solutions.

This is a progress report on the first part of a three year project, the work is not finished and few conclusions can be made at this time.

Z. Mezl

Summary of Progress Report - 1963

University of Montreal

DA-79: Experimental modifications of the permeability of the superficial zone of enamel caries

A method has been developed for observation and measuring of the rate of penetration of staining solutions in initial carious lesions of the enamel:

A ground surface is prepared longitudinally through the tooth and through the center of the lesion. This surface is accurately covered with a transparent varnish, resistant and impermeable to the solutions used. Through the varnish, the degree of penetration of the stain entering through the natural surface of the lesion can be observed with a stereoscopic microscope.

Progress of the penetration of toluidin blue in initial carious lesions was measured.

The permeability of initial carious lesions was compared before and after treatment with the following solutions:

the rehardening solution of Koulourides, Cueto and Pigman
saturated solutions of secondary calcium phosphate in
lactic acid
saturated solutions of dental tissues in lactic acid
lactate buffer solution.

Solutions of calcium phosphate and dental tissues can slow down the penetration of staining solutions in initial carious lesions. Certain lesions easily permeable at the beginning have been rendered impermeable.

Lactate buffer solution at a pH of 5.12 can penetrate in lesions which are relatively impermeable for aqueous solutions.

This is a progress report on the first part of a three year project, the work is not finished and few conclusions can be made at this time.

APPENDIX P

Progress Report

Project DA-109:

Dr. E. T. Pritchard

University of Manitoba

SUMMARY

Sulfolipids of Rat Tissues

The total lipid analysis of the lipids in rat submaxillary glands has been done at one or two age levels. The usual spectrum of lipids was noted. As yet no comment can be made on the relative rate at which these materials accumulate in the growing animal.

In vivo experiments with $S^{35}O_4$ have shown that sulfolipids are labelled in these glands although the label does not tend to persist. This indicates that they undergo a reasonably rapid turnover or replacement. This opposite was found for rat brain sulfolipids whose label persisted for months. Some preliminary results have shown the types of fatty acids associated with the lipid moieties isolated from both brain and gland.

In vitro studies concerning the mechanism of formation of sulfolipids in rat tissues are underway.

Summary of Progress Report

Subject : An X-ray microscopy study of the vascularisation of human dental pulp at various ages.

Grantees : R.L.de C.H.Saunders and H.O.E.Röckert.

Project : D.A. 105.

Objectives

- 1) The development of more satisfactory injection techniques for the preparation of dental pulp vessels for x-ray microscopy.
- 2) The study of the effect of aging on the pulpal vascular pattern.

Methods

- 1) The suction-injection method of aspirating fine particle radiopaque solutions, such as Thorotrast and Micropaque, into the dental pulp vessels of freshly extracted teeth was further modified.
- 2) A micropipette injection method was developed, whereby a micropipette could be inserted through an injection window, placed immediately above the apical foramen, directly into the pulpal vessels. Such injected preparations could be used for both optical and x-ray microscopy.
- 3) Several other methods of injecting dental pulp vessels were explored. These were a) centrifugal injection, b) vacuum impregnation, c) spontaneous injection by capillary attraction, d) chemical methods, whereby it was hoped the results obtained by method 'c' might be enhanced. Insufficient teeth were tested to evaluate these methods.

Continuing Studies

X-ray micrographs are being printed out to determine what age changes can be detected in an age series, using a selected tooth type e.g. canine.

Histological controls are being used to study age changes in pulpal vessels and draw comparisons with x-ray micrograph appearances.

Results

Ninety-eight teeth were injected (partially or completely) by the suction-injection method during the summer of 1963. Despite attempts to improve the suction-injection method, and exploratory experiments with other methods, it has not been possible to significantly increase the percentage of satisfactory pulpal injections. This is unfortunate since it makes it difficult to compare injected teeth and evaluate features in the pulpal vascular pattern.

A report on Micropipette Injection of Dental Pulp Vessels has been completed and submitted for publication.

Regressive changes similar to those reported by Kindlova and Matena (1959, Acta Anat. 37. Supp. 35, 162-692) in the vessels supplying the tip of the rat incisor pulp, have recently been observed in the aging human tooth. Blindly ending arteries, sometimes quite thick, have been repeatedly observed in the tip or cornual region of the pulp, accompanied by disappearance of the adjacent zone of the peripheral capillary plexus of the pulp. These vascular appearances have been interpreted as indicative of regressive changes in blood vessels supplying an area of odontoblasts which have ceased to function.

A 70 page chapter on The Vascular Supply of Dental Tissues has been completed by Saunders and Rockert for inclusion in the forthcoming book on The Structural and Chemical Organization of Teeth (Editors. Richard C. Greulich and A.E.W. Miles: Academic Press 1964).

Prevention of Clotting

As already stated freshly extracted teeth were immediately placed in a sodium citrate solution to minimise intravascular clotting.

The Haematology Laboratory (Mr Sharples) also kindly ran a series of experiments on our behalf to determine the concentration of Thrombolysin (Fibrinolysin) which could break up blood clot in vitro. The cost of using this agent was however deemed prohibitive. Also it deteriorated after two hours of exposure.

In general teeth fell into two groups. One group in which the injectant entered the root vessels, and perfused the pulpal network either totally or partially. And another group in which no sign of injectant could be found in the pulpal vessels, presumably due to clotting in the region of the apical vessels.

A number of preventive measures were tried. Namely warming the injectant, checking the pH of the various solutions to ensure that they were close to the pH of blood, placing the freshly extracted teeth immediately into a vacuum flask containing warm anticoagulant solution, and reducing the time between extraction and injection.

Continuing Studies

a) Teeth, of a selected type, e.g. canine, are being printed out from the x-ray micrographs with a view to observing what age changes can be detected in an age series.

b) Histological studies are being carried out to study age changes in the pulpal vessels and to draw comparisons with the x-ray micrographic appearances.

Results

Ninety eight teeth were injected (partially or completely) by the suction-injection method during the summer of 1963. Despite attempts to improve the suction-injection method, and exploratory experiments with other methods, it has not been possible to significantly increase the percentage of satisfactory pulpal injections. This is unfortunate since it makes it difficult to compare injected teeth and evaluate features in the pulpal vascular pattern.

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Regressive changes similar to those reported by Kindlova and Matena (1959, Acta Anat. 37. Supp. 35, 162-192) in the vessels supplying the tip of the rat incisor pulp, have recently been observed in the aging human tooth. Blindly ending arteries, sometimes quite thick, have been repeatedly observed in the tip or coronal region of the pulp, accompanied by disappearance of the adjacent zone of the peripheral capillary plexus of the pulp. These vascular appearances have been interpreted as indicative of regressive changes in blood vessels supplying an area of odontoblasts which have ceased to function.

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APPENDIX R

SUMMARY OF PROGRESS REPORT ON PROJECT D.A.93

by Dr. J.D. Snouge

Associate Professor of Oral Biology (Oral Pathology), University of Manitoba.

1. Hypersensitivity Reactions in Mucous Membranes. III. Assessment of the minimum amount of various types of allergen capable of producing oral ulceration, and the effect of superimposed local trauma on its ability to diffuse through the mucous membranes.

This work was a continuation of previous experiments carried out to investigate the potential role of hypersensitivity as one possible etiological factor causing recurrent oral ulceration. We have shown that there is a statistically significant increase in the level of recurrent oral ulceration amongst people who tend to suffer from other hypersensitivity diseases and who consequently can be considered as possessing a hypersensitivity 'diathesis'. Similarly, our previous animal experiments have shown that whilst it is relatively difficult in laboratory animals to produce localised hypersensitivity lesions of the mucous membranes due to Immediate hypersensitivity reactions, lesions of the Delayed type can be produced in the oral mucosa almost as easily as on the skin.

The present investigation planned to produce Delayed hypersensitivity in several groups of Guinea Pigs, and then to assess the minimum amounts of Old Tuberculin, Picryl Chloride, and Horse Serum which would produce local ulceration by injection. The inference which it was hoped to draw was that if this amount were relatively small, then other allergens could presumably exist in food and ingested material and possibly diffuse through either the intact mucosa, or defects on its surface, in equivalent concentrations to produce similar lesions. Thus it was also planned that when the animals showed evidence of having become sensitised to the injections, then we would apply allergen to isolated areas of mucosa which had been scraped or scratched - such as might occur due to physiological masticatory trauma - to see whether lesions could be produced purely by diffusion, without having to inject the allergen.

Results obtained show that relatively high concentrations of allergen were required to produce clinical lesions in the Guinea Pig mucosa even by injection. Lesions could be produced by passive diffusion, but these were very superficial in nature, and amounted to erosion, rather than frank ulceration, and were mainly confined to the group of experimental animals previously prepared by cruciate scarification with a Hagedorn-type of cutting needle, rather than the group where the mucosa was firmly scraped with a flat instrument.

It was considered in retrospect that the animals had not become as highly sensitised as those in our previous work. Due to inability to obtain killed Tubercle Bacilli to incorporate into our Freund's Adjuvant - used for initial Delayed sensitisation - we had decided to substitute B.C.G. in the belief that the use of living organisms may indeed enhance the level of sensitisation. Our findings were that not only was the average level of sensitivity achieved much lower, but that in addition it tended to measurably decrease as the experiment progressed. Whilst this produced disappointing results, however, it was a positive finding which may fit in with the clinical fact that the Tubercle Bacillus produces a lot of its damage especially because of this fact that it is capable of inducing a high level of delayed hypersensitivity; whereas the B.C.G. is relatively a-virulent because of its comparative absence of this ability. The work is presently being repeated using our original Freund's Adjuvant, incorporating killed Tubercle Bacilli, and it is hoped to obtain support for this aspect of the work to proceed.

2. Histological Studies of the Effects of Differing Types of Endodontic 'Splints' on the Apical Tissues of Dogs' Teeth.

Clinical reports have been made claiming success for the splinting of periodontally involved teeth by passing metal posts through the root canals into the apical bone. This method obviously possesses great advantages in that it does not increase oral stagnation in the area, such as external splinting methods would do, because this very stagnation may have been an original contributory factor to the production of the periodontitis which loosened the tooth.

Posts consisting of Chrome Cobalt, Acrylic, Ivory and Dentine were passed via the root canal, left for 1 month and 3 month periods, and then the specimens were examined histologically.

The Ivory and Dentine posts were physiological compatible with the tissues, and not only was there complete absence of Foreign-Body rejection, but bone was deposited along a great deal of their surface to produce clinical ankylosis. Chrome Cobalt and Acrylic posts showed no evidence of frank rejection, but there was minimal chronic round-cell infiltration in adjacent regions, but bone appeared to be existing quite happily in the adjacent regions, though separated from the metal by a dense 'sac' of fibrosis.

Mature consideration would make it appear that a fibrous reaction of surrounding tissues is preferable to ankylosis, as it would give some measure of mobility, whilst still premitting a degree of support to be obtained. Thus the tooth may derive support from two sources - the post and the periodontal membrane, whereas with ankylosis only the post gives support. One further feature was that the Ivory post, and the associated tooth apex showed some evidence of resorption which may have produced significant weakening if such a device were used clinically.

3. Histological Studies of the Effects of Gold Foil preparations upon the pulp and periapical tissues of dogs' teeth.

Despite the emotional arguments associated with the virtues of gold-foil restorations, the large volume of literature published concerning them consists mainly of clinical impressions, and little scientific histopathological investigations have been carried out on the effects which they produce.

Pilot studies were done on this subject, and a method devised whereby the pulpal tissues could be fixed and preserved efficiently, whilst still retaining the histological relation of the apical tissues. The general impression obtained supported the limited findings of James and Schour (1955) that marked pulpal damage resulted, associated with a high degree of bone resorption around the periodontal membrane. It is considered that this study should be continued and sufficient specimens obtained to give statistical validity to a procedure which unavoidably cannot be standardised. Similarly we would like to continue the studies of the pulp over a longer period to see the proportion of change which is reversible.

4. A survey to assess the amount of tap-water drunk by children during the period of active enamel formation.

A survey was conducted to assess the amount of tap-water drunk by children in general, and particularly those in urban compared with rural areas, and those of higher income families compared with lower income groups. Simultaneously a survey was carried out on skull X-Rays to assess the exact state of development of the enamel of teeth belonging to children of this age.

It was found that tooth development coincided mainly with previously published results, although the development of the bicuspid teeth tended to be quite considerably later than usually assumed to be the case. The average water-consumption, including that incorporated into solid foods during cooking, was approximately 30% of total intake, for a child up to 5 years. The bulk of the remainder consisted of milk. Water intake tended to be higher in Rural dwellers than in Urban, and higher in children of lower income groups than of higher income groups, although the statistical validity of our findings has not yet been fully assessed.

These findings would seem to imply that if ingested fluorine is actually incorporated into tooth-substance to produce increased caries-resistance during enamel development, then public health workers would be better advised to press for 'fortification' of milk, as a great deal more of this is taken by the child. Even if cows are fed a high-fluorine drinking water the level of fluorine in their milk rarely rises beyond 0.3 p.p.m., and thus the milk would require direct addition of fluoride. Another possible implication of these findings is that the resistance undoubtedly associated with fluorination of water derives not from incorporation of fluorine in the developing tooth so much as due to local action either on the enamel, or the bacterial-plaque-^{carbohydrate} ~~glucose~~ complex.

It is desired to extend this survey to include greater numbers for increased validity of findings.

(A below)

During the Inter-region, we carried out some experiments with Dental students, who volunteered as subjects to permit studies of the effect of ingested BaF_2 on salivary phosphorus levels. (See 2 below)

A. Ingested Phosphate and Rat Salivary Phosphorus.

Methods

Adult male Sprague-Dawley rats were used for experiments this year. They were housed in groups of ten in stainless steel cages. They were maintained on a diet of Purina Chow Chunks and water *ad libitum*, unless indicated otherwise in this report.

In order to obtain saliva samples, the mouth of the animal was held open with a device made in our laboratory for this purpose. Essentially, this meant that a wire trap was hooked over the upper incisors of the rat and the mouth was opened sufficiently to expose the tongue, and no injury resulted either to the gums of the animal or the fingers of the person preparing the animal to receive the phosphate solution. Three #1 dental cotton pellets were placed under the tongue with fine, hand forceps and left for about two minutes until the pellets were wet. The average cotton pellet weighed 3 - 4 mm. and had three absorbed 30 - 40 mm. volume.

Annual Progress Report for 1963.

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Grantee - Jules Tuba, Faculty of Dentistry, University of Alberta.

Topic I - The Influence of Ingested Disodium Phosphate on the Phosphorus Content of Saliva in: (A) the Rat, and (B) the Human. (With the assistance of Mrs. E. Bekesi)

Introduction.

A year ago we reported on the first experiments wherein we determined rat salivary phosphorus levels. These values were established for 2 small groups of adult male, Sprague-Dawley rats: a normal, unfasted group and a group of animals fasted over night and then force-fed Na_2HPO_4 (Pi) solution.

Following our report of a year ago, our colony of rats was wiped out by a respiratory infection, and it took us a while to get the animal room disinfected, and the colony re-established and continue our experiments. (A below)

During the inter-regnum, we carried out some experiments with Dental students, who volunteered en masse to permit studies of the effect of ingested Na_2HPO_4 on salivary phosphorus levels. (See B below)

A. Ingested Phosphate and Rat Salivary Phosphorus.

Methods

Adult male Sprague-Dawley rats were used for experiments this year. They were housed in groups of ten in stainless steel cages. They were maintained on a diet of Purina Chow Checkers and water ad libitum, unless indicated otherwise in this report.

In order to obtain saliva samples, the mouth of the animal was held open with a device made in our laboratories for this purpose. Essentially, this means that a wire loop was hooked over the upper incisors of the rat and the mouth was opened sufficiently to expose the tongue, and no injury resulted either to the gums of the animal or the fingers of the person preparing the animal to receive the phosphate solution. Three #3 dental cotton pellets were placed under the tongue with fine, bent tweezers and left for about two minutes until the pellets were wet. The average cotton pellet weighed 5 - 7 mgm. and 3 of these absorbed 50 - 80 mgm. saliva.

The weight of saliva was determined by the difference in weight of the wet and the dry cotton pellets. The 3 pellets used for each animal were dropped immediately after collection of saliva into 15 x 125 mm. Pyrex tubes to minimize loss of weight by evaporation.

The pellets were then wet-ashed. To each tube was added one ml. 5M H_2SO_4 . In order to have sufficient P present to give reliable optical density readings on the spectrophotometer, we added to each sample, 1 ml. of P standard (0.05 mgm. P/ml.). The sides of the tubes were washed down with one ml. water. Each tube and its contents plus 2 glass beads were heated on a boiling water bath until fumes appeared. By this time the pellets were disintegrated and the solution was dark brown or black; this facilitated the subsequent digestion on a micro-Kjeldahl digester at a moderate temperature. The charred contents of the tubes were treated with 5 drops of 30% hydrogen peroxide and heated on the digester until sulphuric fumes appeared and the digest cleared. Control tubes were used with pellets only to determine P content. To the clear liquid in each tube was added 3 - 4 ml. water, rinsing the sides of the tube at the same time. The addition of 2.5 ml. of 2.5% ammonium molybdate solution with mixing, was followed by 1 ml. of aminonaphthosulfonic acid reagent. After mixing, the contents of the calibrated tube were diluted with water to the 10 ml. mark. This volume was the minimum we could use without crystallization (perhaps of ANSA) occurring. The blue colour was read in the Beckman DU Spectrophotometer within 10 minutes at 660 mu against a reagent blank. A standard (in duplicate or triplicate) containing 0.05 mgm. P/ml. was used with each group of tubes.

Note that the above method for determining P permits use of one tube only and avoids loss of P by transfer from one tube to another.

Force-feeding Na_2HPO_4 Solution

Saliva was obtained from a group of ten rats which had food before them continuously. Another group of ten animals was fasted over-night (20 hours) before saliva was taken from them for P analysis. Then a group of unfasted animals and another group fasted for 20 hours (10 animals per group) were force-fed one ml. of $Na_2HPO_4 \cdot 7H_2O$ containing 23 mgm./ml. P. The feeding was performed with the aid of a syringe and #17 needle. The 5 cm. needle was blunted at the sharp end with a bead of solder, and bent slightly at the blunted end to facilitate passage of the needle down the esophagus to the stomach. The phosphate solution was fed in 2 one-half ml. portions; each half ml. within about 3 or 4 minutes of the other. Saliva samples were obtained before feeding and one hour later. An additional fasted group of 10 animals was fed as above, but samples were obtained at 0, 1, and 2 hours.

The effect of feeding double the above dose of P was also examined. The animals (10) were force-fed two one-ml. quantities of the same P solution as above (a total) of 46 mgm. of P. Samples were obtained at 0, 1, and 2 hours.

Results

These are given in Table I.

Saliva was contaminated by residual food particles to varying degrees in Groups I and III and phosphorus values show a wide range and large standard error of the mean in both groups. In all samples from Groups IV, V, VI, and VII, the phosphorus content was significantly elevated compared with the control group (II), ($P < 0.01$). The significance of these results is emphasized by the number of saliva samples analyzed from each group.

The concentration of $\text{Na}_2\text{HPO}_4 \cdot 7\text{H}_2\text{O}$ in aqueous solution was 20.5% because this was a concentrated solution, but one which did not tend to crystallize out in the refrigerator. It also gave a reasonably high dosage of 23 mgm. P/ml. The salivary values of P in the 2 groups which were given a dosage of 46 mgm. P in 2 ml. reflect the lack of equilibrium in the gut of these rats. They displayed a marked gastrointestinal peristalsis, presumably induced by the larger volume and amount of phosphate, since it is known that the gut has a limited tolerance for Na_2HPO_4 . An optimal dosage of P appears to be around amounts fed to Groups IV and V under the conditions of our experiments.

Conclusions

The data in the Table indicate that in fasted rats, force-fed phosphate produced significant elevation of the total salivary P levels.

There could be some value in feeding adult male rats synthetic diets with various levels of phosphate. However, the results could be obscured by the presence of substances in the diet.

* Numbers in brackets under average values give numbers of samples from each group.

TABLE I

THE EFFECT OF FORCE-FEEDING $\text{Na}_2\text{HPO}_4 \cdot 7\text{H}_2\text{O}$ SOLUTION
TO ADULT MALE RATS

Average values are given for groups with standard deviations (SD) and standard errors of the means (SE).

Group	Average salivary P (mg %)	SD	SE	Probability	Remarks
I. Unfasted	13.80 (30)*	± 8.35	3.87	-	-
II. Control (fasted 20 hrs)	8.99 (68)*	± 2.76	0.33	-	-
III. Force-fed (23mg P) (unfasted)	17.24 (26)*	± 12.12	5.39	-	1 ml of $\text{Na}_2\text{HPO}_4 \cdot 7\text{H}_2\text{O}$ per rat. Saliva sample taken 1 hour after force-feeding.
IV. Force-fed (23mg P) (fasted 20 hrs)	16.63 (26)*	± 8.42	1.65	$\underline{P} < 0.01$	1 ml of $\text{Na}_2\text{HPO}_4 \cdot 7\text{H}_2\text{O}$ per rat. Saliva sample taken 1 hour after force-feeding.
V. Force-fed (23mg P) (fasted 20 hrs)	13.15 (34)*	± 5.51	0.95	$\underline{P} < 0.01$	1 ml of $\text{Na}_2\text{HPO}_4 \cdot 7\text{H}_2\text{O}$ per rat. Saliva sample taken 2 hours after force-feeding.
VI. Force-fed (46mg P) (fasted 20 hrs)	12.46 (41)*	± 5.42	0.85	$\underline{P} < 0.01$	2 ml of $\text{Na}_2\text{HPO}_4 \cdot 7\text{H}_2\text{O}$ per rat. Saliva sample taken 1 hour after force-feeding.
VII. Force-fed (46mg P) (fasted 20 hrs)	19.52 (46)*	± 8.36	1.23	$\underline{P} < 0.01$	2 ml of $\text{Na}_2\text{HPO}_4 \cdot 7\text{H}_2\text{O}$ per rat. Saliva sample taken 2 hours after force-feeding.

* Numbers in brackets under average values give numbers of samples from each group.

(1) Shuman, J.L., Tabell, F.H., Gibson, W.A., and others, J. Inorganic Nutrition, 1963, 1962.
(2) Molare, F.J. Further Studies on the Carcinogenic Effect of Organic and Inorganic Phosphates, J. Natl. Canc. Inst., 42: 593, 1963.

Topic I

B. Ingested Phosphate and Human Salivary Phosphorus.

Introduction

There has been considerable evidence that dietary phosphate supplements reduce the caries incidence in experimental animals. It has not been shown to the satisfaction of all interested in these studies whether this cariostasis is developmental, post-developmental, local or systemic (1) (2).

In Part A, Topic I, of this report, studies with the rat indicate that a systemic effect may be involved. Shannon et al (1) reported studies with humans in which they attempted to relate caries experience to serum and saliva Pi concentrations.

In our laboratory we have studied the effect on human salivary P of Na_2HPO_4 ingested in a fasting state and after food was consumed.

Experiment I

A class of 40 dental students was divided into 4 groups of 10 each. The students had breakfast before 8:00 a.m., but they had no lunch and did not eat until after the experiment ended at about 3:00 p.m. They were asked not to drink tea or coffee, and to refrain from smoking (if possible) during the time from 8:00 a.m. to 3:00 p.m.

Method

A sample of unstimulated saliva was obtained from each student at time zero, by placing 2 size #2 dental cotton pellets under the tongue. Thereafter the students ingested as follows:

Group I - an empty gelatin capsule each.

Group II - a gelatin capsule containing 0.3 g. Na_2HPO_4 .

Group III - a gelatin capsule containing 0.6 g. Na_2HPO_4 .

Group IV - a gelatin capsule containing 0.1 g. Na_2HPO_4 .

Samples of saliva were obtained one hour and two hours after ingestion.

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- (1) Shannon, I.L., Isbell, G.M., Gibson, W.A., and O'Leary, T.J. Inorganic Phosphate Concentration in Body Fluids as Related to Dental Caries Status, J. Dent. Res., 41: 1373, 1962.
 - (2) McLure, F.J. Further Studies on the Cariostatic Effect of Organic and Inorganic Phosphates, J. Dent. Res., 42: 693, 1963.

P content of saliva samples was determined by wet-ashing as above (A) and by the colorimetric method of Fiske and Subbarow as described above. Recovery experiments of standard P solution with dry pellets showed that no loss of P occurred during ashing and colour development.

Results

The salivary P values indicated that it would be best to feed the fasted students 0.6 g. Na_2HPO_4 in gelatin capsules, and that the most pronounced elevation of P occurred after 2 to 3 hours. The average salivary P values for students receiving empty capsules differed in 2 hours by -0.87 mgm.%, whereas the group who ingested capsules containing 0.6 g. Na_2HPO_4 had an average increase in salivary P of +1.65 mg./% or a net change of about 2.5 mg./%.

Experiment II

The class of 40 students was divided into 2 groups. The students had fasted from breakfast until the end of the experiment, about 3:00 p.m. Group I ingested empty capsules. Group II received gelatin capsules containing 0.6 g. Na_2HPO_4 . Saliva samples were obtained at 0, 1, 2, and 3 hours from both groups. 200 - 300 mgm. saliva samples were collected by means of cotton pellets from each donor.

In Group I, which ingested empty gelatin capsules, the average salivary P levels fell 2 mg.% in 3 hours. In the second group the zero hour average value was not only maintained, but slightly elevated by the 3rd hour (about 0.25 mg./%). Hence, the net effect of ingested P was about the same as in the first experiment.

Individual salivary P levels seemed generally characteristic of a student in several experiments under the conditions of our experiment. In addition, it was observed that response to ingested Na_2HPO_4 was maximal in some cases in 2 hours and in some cases in 3 hours. This was borne in mind for the next experiment.

Experiment III

The Effect on Human Salivary Phosphorus Levels of Na_2HPO_4 in Gelatin Capsules Ingested With and Without Food.

The class of 40 dental students were fasted as in previous experiments. (These experiments are a testimony to the good nature and enthusiasm of the class of 1965.) The experiments went on for six hours

this time to test the effect of (1) food and (2) a second capsule of Na_2HPO_4 at the end of 3 hours.

Samples of saliva were obtained at the beginning of the experiment from all the students (zero hour). With 4 people collecting samples this took a few minutes only, especially since the students were now highly conditioned to the procedure.

A. The class was divided into 2 equal groups. One group was given empty gelatin capsules plus a drink of water, and a second empty capsule 3 hours later. Samples of saliva were taken at 0 hours, 2 hours, 3 hours (just before second capsule), 5 hours and 6 hours. The second group was given one gelatin capsule containing 0.6 g. Na_2HPO_4 per person and a second similar capsule 3 hours later. Saliva was sampled as with the first group.

B. The class was divided into 2 equal groups. One group was given food, plus an empty capsule after the food and another empty capsule 3 hours later. The second group was given food, plus 0.6 g. Na_2HPO_4 in a capsule after the food and a second similar capsule 3 hours later. The food was simple. Each student was given one tomato-lettuce sandwich on white bread, without butter or dressing, plus a paper cup full of apple juice. We found the menu delicious: i.e., better than no food. Saliva was again taken at 0 hours (then food and capsule), 2 hours, 3 hours (then second capsule), 5 hours and finally 6 hours after the experiment began.

This represents a very large number of weighings, which had to be prompt and accurate to the third (if not the fourth), decimal place in grams. It is pleasant to record that this all went off without mishap.

The P in the diet per student we estimated to be about 120 - 140 mgm. P in 0.6 g. Na_2HPO_4 fed in capsules amounted to 127 mgm. The amount of inorganic P in capsules was not increased above this level to avoid any possible effect on intestinal peristalsis in fasting individuals.

Results

These are given in Table II as mean P values in mgm./% with standard deviations and standard errors of the means. Statistical analyses for t values yielded 3 instances in which $P < 0.05$, that is, significant differences were demonstrated in salivary P levels.

The students who were fasted during the six hour experimental period and at least 4 additional hours since breakfast showed a fall in P levels if they received only empty capsules. This fall verges on significance.

TABLE II
THE EFFECT ON HUMAN SALIVARY PHOSPHORUS LEVELS OF Na_2HPO_4 IN GELATIN CAPSULE
INGESTED WITH AND WITHOUT FOOD

Means are given with standard deviation (SD) and standard error of mean (SE)

Time saliva samples taken	Fasted 4 hours and given no lunch						Fasted 4 hours and given lunch at 0 hour					
	Empty gelatin capsule			0.6 g Na ₂ HPO ₄			Empty gelatin capsule			0.6 g Na ₂ HPO ₄		
	mg% P	SD	SE	Mg% P	SD	SE	mg% P	SD	SE	mg% P	SD	SE
0 hour (1st capsule fed)	16.42	±3.68	0.87	16.12	±2.62	0.68	16.30	±4.34	0.99	15.95	±4.05	0.93
2 hours (after 1st capsule)	A* 15.64	±3.05	0.72	16.20	±4.64	1.06	A* 18.25	±4.07	0.91	16.21	±3.80	0.87
Second gelatin capsule was given												
3 hours (after 1st capsule)	15.86	±2.97	0.74	17.41	±4.37	1.00	B* 18.40	±4.80	1.05	B* 15.18	±4.13	0.92
5 hours (after 1st capsule)	15.64	±4.07	1.02	17.97	±5.31	1.25	15.86	±4.32	0.99	17.64	±6.11	1.37
6 hours (after 1st capsule, 3 hours after 2nd capsule)	C* 15.22	±1.74	0.44	C* 18.15	±4.53	1.07	15.40	±4.69	1.02	15.27	±3.94	0.88

Pairs of values compared are indicated by paired letters: (A-A), (B-B), (C-C).

* P less than 0.05

The other group of similar hungry students who were given 0.6 g. Na_2HPO_4 in gelatin capsules at 0 and 3 hours showed a rise in salivary P levels. The P levels at the end of the six hour experiments showed a significant difference between these two groups (A - A). That is, not only was the fall of P prevented in the second group, but an actual elevation occurred in P levels which can only be attributed to the ingestion of Na_2HPO_4 in gelatin capsules, (accompanied always with a drink of water to avoid any possibility of local effects).

The various trends in these 2 groups could be sharpened by calculating the mean differences of paired results and the standard error of these mean differences. This, in fact, will be done before publication is attempted.

The third group in the Table, who were fed empty gelatin capsules and who received a sandwich and apple juice, showed a significant rise in salivary P levels at 2 hours when compared with the fasting group. This rise was maintained until 3 hours and could be attributed to absorption in the gut of P from the modest lunch eaten by the students.

The values of P for the group fed 0.6 g. Na_2HPO_4 after eating lunch were unexpected and surprising. The food and phosphate together had no effect on salivary P for 3 hours. However, when food was fed alone it elevated salivary P levels during the same 3 hours. One must assume some inhibitory effect of P above certain concentrations on absorption from the gut. This actually was what we noted in a preliminary experiment (Experiment I) with students fed 1.0 g. Na_2HPO_4 , but with a relatively small number of students.

It has been noted by the grantee (1) and by Triantas (2) that variations in pH of environment of tissues or cells could affect utilization of P_i and alkaline phosphatase. It is also possible that $[\text{Mg}^{++}]$ can be influenced in these experiments, thereby influencing P_i absorption. Finally, there is a strong possibility that constituents of the food inhibit P_i absorption.

Conclusions

There is evidence that P_i can be absorbed from the gut of the fasting human and that it appears in the saliva.

This absorption is modified by food consumed immediately prior to the ingestion of P_i .

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- (1) Tuba, J. - Unpublished data.
 - (2) Triantas, E. - Ph.D. Thesis.

Additional Experiments

In the next 3 months some of the above experiments will be repeated with students. Experiments with rats will be repeated and estimations will be made of serum and intestinal Pi, of serum and intestinal alkaline phosphates, and of serum and intestinal Mg.

Topic II - The Influence of Force-fed Calcium Chloride on Calcium Levels in Liver and Intestinal Tissues of the Rat. (With the assistance of Mrs. Eva Schenk and John F. Milner.)

Introduction

Jenkins (1) remarks that "Calcium and phosphate are among the ions which are poorly absorbed, and many factors influence the process, some favouring and others hindering it." "The absorption of neither ion can be considered alone."

Our work on the absorption of phosphate from the gut is discussed under Topic I of this report. Certainly the first part of Jenkins' quotation applies to our findings with phosphate. As it was tempting to consider the second part of the quotation and to put the legions of those who have studied the absorption of calcium from the gut. The fashion, at the moment, is to use Ca^{45} for absorption studies. As a rule, the amount of Ca^{45} absorbed is estimated. Our concern, however, was to study the influence of force-fed calcium chloride on the calcium content of the gut, liver and blood.

Methods

Adult male Sprague-Dawley rats were used for these experiments. They were housed and fed as described in Topic I.

Animals were heavily anesthetized with nembutal, and the abdomen and chest were quickly cut open. Blood was removed from the heart with a heparinized syringe and needle. This complete exsanguination expedited the demise of the animal. The blood was transferred at once to a heparinized tube and centrifuged later. The liver was removed next, and then the gut from the pylorus to the ileocecal junction. The gut was washed with normal saline, cut into 4 segments, drained of excess water, and blotted with filter paper. The proximal quarter of the small intestine, which included the duodenum, and the distal quarter, i.e., proximal to the ileocecal junction were used in our studies.

(1) Jenkins, G.N., "The Physiology of the Suck", 1963. Springfield - Thomas

Topic II - The Influence of Force-fed Calcium Chloride on Calcium Levels In Liver and Intestinal Tissues of the Rat. (With the assistance of Mrs. Eva Bekesi and John F. Milner.)

Introduction

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The tissues were wet-ashed in our earlier work in the manner described in Project I. Calcium was precipitated as calcium phosphate. Pi was estimated by the Fiske and Subbarow method (2) and thence the value of calcium was calculated. Calcium in blood plasma was estimated with oxalate and permanganate titration (3).

We found this method to be reliable, but slow. Eventually, we changed to dry-ashing the tissues. Sections of the intestine and of the liver were heated in platinum crucibles at 1800°F for 3 - 6 hours. The ash was dissolved in 3 drops of concentrated HCl. The solution was adjusted to pH of around 3 with NaOH and the volume was increased with water to 5 ml in pyrex test tubes. Aliquots of 0.1 ml. of the final solution were used for liver samples and 0.05 ml. for intestine samples to determine the calcium content. In the case of blood plasma, 0.05 ml. aliquots were used.

Calcium was determined by means of an Oxford Titrator Model 301. This is based on titration with a chelating agent, ethylenediamine tetraacetate in an alkaline medium. The method is similar to that of Bett and Fraser (4). An aliquot of the tissue solution or serum (see above) was placed in a plastic disposable cup. A drop of 6.25 N KOH was added and a drop of the indicator solution, calcein, with swirling to mix. Titration with Na EDTA is carried out under ultra-violet light, until the yellow-green fluorescence in the cup is quenched. 0.05 ml. of standard Ca solution was used with each group of determinations. Recovery determinations gave 96-97% values. Titration of each tissue solution and of blood was performed at least in duplicate.

Calcium was determined in the proximal quarter and distal quarter of the small intestine of normal, unfasted animals. Mean values for the proximal fraction were 11.64 mg.% (S.D. = 2.28 and S.E. = 0.76) and for the distal quarter, 9.96 mg.% (S.D. = 1.91 and S.E. = 0.67). It was much more difficult to use the distal quarter of the small gut in non-fasted animals, because of the solid material therein, than in rats deprived of food for 48 hours. Hence, this group must be repeated again, and is not at this time included in Table I of Topic II.

Other animals in this investigation were deprived of food, but not of water, for 48 hours. Force-feeding was carried out as described in Topic I and in last year's progress report. If rats are maintained on a synthetic diet which contains 4% McCollum-Davis salt mixture, we estimated that they would consume 0.8 - 1.0 g. salt mixture. On this

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- (2) Roe, J.H., and Kahn, B.S. (1929), J. Biol. Chem., 81, 1.
(3) Clark, E.P., and Collip, J.B. (1925), J. Biol. Chem., 63, 461.
(4) Bett, I.M., and Fraser, C.P. (1958), Biochem. J., 68, 13P.

basis the amount of Ca^{++} ingested would be between 80 - 90 mgm. Harrison and Harrison (5) in their studies on rat intestinal absorption of Ca^{++} , used 1 ml. of a solution containing 10 mg. Ca^{++} . Others have used varying amounts, but we decided to use a force-feeding of 10 mg. of Ca^{++} . This amount of calcium ion is contained in 1 ml. of a 2.7674% solution of CaCl_2 . Force-feeding of calcium chloride was carried out as described above in Topic I. One ml. CaCl_2 solution was force-fed in 2 one-half ml. portions, (one after the other in 2 - 3 minutes) to animals fasted 48 hours. This was repeated with another group of rats, using double the concentration of CaCl_2 (5.5348%). Force-feeding lactose alone also lactose together with CaCl_2 . Vaughan and Filer (6) reported the results of investigations of the enhancing action of certain carbohydrates on intestinal absorption of calcium in the rat. The carbohydrates included lactose. Vaughan and Filer speculated on the reasons for their findings. Their findings are elucidated, certainly in the case of lactose by the observations of Charley and Saltman (7). Charley and Saltman pointed out that no theories adequately explain the effect of sugar on gastrointestinal absorption of calcium. These theories have been based on studies with intestinal microflora, pH of the intestinal tract, and higher osmotic or hydrostatic pressures induced by the sugars. The work of Charley and Saltman proved that chelation of calcium with lactose occurred in vitro. They suggested that the formation of such a soluble complex offers an explanation for previous results of nutrition studies. It is still unknown whether the complex is transported through the membranes of the gut or if they maintain Ca^{++} in a form readily available for transport.

These ideas concerning Ca^{++} transport are of interest to us not only because of the work described in this report. We are carrying out studies at this time, under a grant from the Medical Research Council of Canada, the β -galactosidase of the gut. Some aspects of studies of lactose hydrolysis in the gut under various experimental conditions should shed light on the overall problem of Ca^{++} absorption.

In the β -galactosidase experiments we force-fed 6% lactose to fasted rats. In our experiments on calcium absorption, reported here, we force-fed 6% lactose, with and without 2.7674% CaCl_2 . In the former instance, lactose and CaCl_2 were contained in the same solution.

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- (5) Harrison, H.E., and Harrison, H.C. (1951), 188 . 83.
 - (6) Vaughan, O. W., and Filer, L.J. Jr. (1960), 71 . 10.
 - (7) Charley, P., and Saltman, P. Science (1963) 139 . 1205.

Results

These are given in Table I, of Project II. No significant variations were observed in levels of plasma Ca^{++} in the groups subjected to 5 different experimental treatments.

The $[\text{Ca}^{++}]$ in liver was significantly affected by force-fed CaCl_2 . There is no difference in mean values for the 2 groups which received 10 mg. and 20 mg. Ca^{++} . This was not surprising because it was found that animals which were fed the higher amount of CaCl_2 had severe diarrhea and a great deal of fluid was found in the gut during dissection.

The proximal quarter of the small intestine, i.e., contiguous to the pylorus, showed no significant variation in $[\text{Ca}^{++}]$ in any of the 5 groups. This is what one could expect in view of the observations of Vaughan and Filer. They found that absorption of Ca^{++} in rat duodenum was not enhanced by sugars but absorption from ligated ileal segments was much increased. Sallis and Holdsworth (8) claimed that increased absorption of Ca^{++} in the distal small intestine occurs in vitro with everted gut sacs, but this is not true in vivo where absorption is uniform along the whole small intestine. It is to be noted that their work and that of Harrison and Harrison was carried out with rachitic animals. There are, then, various interpretations of results by different research groups.

Several groups seem agreed on (1) the need for fasting animals, to avoid effects of residual dietary Ca^{++} or inhibiting substances and (2) that maximal removal of Ca^{++} from the ileum is obtained within 4 hours after feeding. It seems that our practice of fasting the rats for 48 hours and killing them 3 hours after force-feeding is a reasonable one.

The results in Table I show a highly significant increase in $[\text{Ca}^{++}]$ in the distal quarter of the small intestine for the groups fed CaCl_2 alone. It should be recalled that the Ca^{++} values in the first and fourth quarters of the intestine of animals on the standard laboratory diet were not significantly different from corresponding values for fasted animals. In other words the ordinary diet did not appreciably affect Ca^{++} levels in the gut. Also since there was no rise in Ca^{++} levels in the proximal gut in any group, the rise in the distal gut was a real one, and not one to be associated with adherent Ca^{++} . This is further confirmed by the significant rise in the Ca^{++} content of the livers.

The addition of lactose to the CaCl_2 solution resulted in a lower but still significant rise in $[\text{Ca}^{++}]$ in the distal quarter of the intestine. On the basis of the observations of Vaughan and Filer, also

(8) Sallis, J.D., and Holdsworth, E.S. Am. J. Physiol. (1962), 203, 1962.

of Charley and Saltman, one may assume that chelation of Ca^{++} with lactose has occurred and the rate of Ca^{++} absorption has been stimulated. This could account for the changed $[\text{Ca}^{++}]$ in liver.

Force-fed lactose alone had no significant effect on any of the tissues studied.

Conclusions

1. The force-feeding of CaCl_2 solution to 48-hour fasted rats effected a significant rise in the $[\text{Ca}^{++}]$ of the distal quarter of the small intestine and the liver.
2. The simultaneous feeding of lactose decreased the magnitude of this change in the distal small intestine, although it was still statistically significant. The increase of $[\text{Ca}^{++}]$ in liver was no longer significant.
3. It is assumed that the modifying effect of lactose is due to chelation of Ca^{++} , and to stimulation of the rate of absorption.
4. The force-feeding of lactose, in the absence of Ca^{++} from the solution, had no significant effect on $[\text{Ca}^{++}]$ in the gut or the liver.

The Effect of Force-fed CaCl_2 on Calcium Content of Liver, Intestine, and
Blood Plasma in Fasted Rats

Table I

Mean values for Calcium are given in mg/% wet tissue with standard deviation (SD) and standard error of mean (SE)

Groups	Number of rats	Blood plasma			Liver			Proximal quarter of small intestine			Distal quarter of small intestine		
		mg% Ca	SD	SE	mg% Ca	SD	SE	mg% Ca	SD	SE	mg% Ca	SD	SE
48 hours fasted (control) as were other groups	20	9.85	± 0.08	0.03	3.67	± 0.14	0.03	11.44	± 1.61	0.39	11.27	± 2.06	0.50
1 ml of 2.7674% CaCl_2 (10 mg Ca^{++})	15	9.53	± 0.34	0.09	4.36*	± 0.47	0.12	11.13	± 1.33	0.37	17.76*	± 4.02	1.11
1 ml of 5.5348% CaCl_2 (20 mg Ca^{++})	15	9.71	± 0.18	0.05	4.58*	± 0.33	0.08	12.03	± 1.41	0.38	15.67*	± 2.43	0.65
1 ml of 2.7674% CaCl_2 -6% Lactose	10	9.28	± 0.33	0.10	3.95	± 0.39	0.14	10.68	± 2.04	0.72	13.55 ^o	± 2.00	0.71
1 ml of 6% Lactose	10	9.42	± 0.20	0.06	3.45	± 0.32	0.10	11.23	± 2.52	0.80	12.59	± 1.25	0.40

* P less than 0.01
° P less than 0.02 compared with control values

Methods

Gingival tissue was supplied to us by the Dental Clinic, through the kindness of the Departments of Periodontia and Oral Surgery. Immediately after gingivectomy, the tissue was rinsed in normal saline to remove clotted blood as much as possible. The tissue was stored in 95% ethanol, usually for several days and kept in a refrigerator.

Topic III - N-Acetyl Neuraminic Acid in Normal and Diseased Gingival Tissue. (With the assistance of Mrs. Eva Bekesi.)

Introduction

During the past three years the grantee has directed and been associated with studies of protein-bound carbohydrates in the plasma of normal and tumour-bearing humans and rats (1). The investigations led to development of techniques for analyses of various protein-bound carbohydrates in tissues.

Six protein-bound carbohydrates are studied in various mucoproteins: D-glucosamine, D-galactosamine, L-fucose, N-acetylneuraminic acid, D-glucose, and D-galactose. These proteins are referred to as glycoproteins or mucoproteins; sometimes indiscriminately. West and Todd (2) quote Karl Meyer's definition of a mucoprotein, viz; a protein-bound carbohydrate which contains over 4% hexosamine. Others distinguish glycoproteins and mucoproteins on the nature of the linkage between the prosthetic group and the polypeptide chain.

Mucoproteins have been studied in human and animal saliva (3). Neuraminic acid determination has proved a valuable indicator of the mucoprotein content of chronic bronchitis sputum in the human. High concentrations of neuraminic acid have been reported in bovine and ovine submaxillary gland mucoprotein.

The finding of neuraminic (sialic) acid was reported in human gingival tissue from patients with chronic periodontitis (4). Because carbohydrate protein complexes form an important part of extra-cellular ground substance it seemed to us that these could be affected by a number of periodontal diseases. In addition to local disease there is a strong probability that systemic factors could produce changes in carbohydrates of gingival tissue, e.g.: diabetes mellitus or experimental diseases.

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- (1) Macbeth, R.A., Bekesi, J.G., and Tuba, J. Cancer, (1963) 23 . 938.
 - (2) West, E.S., and Todd, W.R. "Textbook of Biochemistry" (1962) Macmillan, New York.
 - (3) Stacey, M., and Barker, S.A. "Carbohydrates of Living Tissues", (1962), Van Nostrand, Toronto.
 - (4) Thonard, J.C., and Blustein, R., Int. Assoc. for Dent. Res. Abstracts, (1963), page 47.

Methods

Gingival tissue was supplied to us by the Dental Clinic, through the kindness of the Departments of Periodontia and Oral Surgery. Immediately after gingivectomy, the tissue was rinsed in normal saline to remove clotted blood as much as possible. The tissue was stored in 95% ethanol, usually for several days and kept in a refrigerator.

Before analysis the tissue was rinsed 3 times with 95% ethanol, blotted with filter paper, and left for a few minutes to dry. It was then homogenized with about 20 ml. of 2:1 acetone-ether mixture in a Virtis homogenizer for 5 - 6 minutes. The homogenate was centrifuged at 3,000 r.p.m. for 5 minutes and washed twice with acetone-ether. After the last washing was decanted and the pellet was drained, 10 ml. 0.1 N H_2SO_4 was added, and mixed with the solid material. This was left over night at room temperature, centrifuged in the morning. After decanting the contents of the tube were drained. The solid material was placed on a watch glass, an aliquot was removed for nitrogen estimation and the remainder was weighed. The weighed sample was then transferred back to the centrifuge tube and after the addition of 10 ml. of 0.1 N sulphuric acid, the tissue residue was hydrolyzed for an hour at $80^\circ C$ on a water bath. The hydrolyzate was allowed to cool to room temperature and centrifuged. The supernatant fluid was decanted, the previous hydrolytic step was repeated once and the supernatants were combined to give a total of about 20 ml. of liquid from the 2 hydrolyses. The supernatant was then passed through a column.

Preparation of Columns

About 100 g. of an anion exchange resin (AG1-X8 : 200-400 mesh, formate form) was placed in a 400 ml. beaker. After the addition of about 300 ml. of 2 N HCl, the beaker and contents were heated at $85^\circ C$ for an hour. Then the liquid was decanted and the procedure was repeated once more, to ensure conversion to the chloride form. After the HCl was decanted once more, distilled water was added; the resin was filtered on a Buchner funnel and washed with distilled water until the washings are near pH 7.0. The washed resin was placed in a beaker with about 200 ml. 2 N sodium acetate and left for an hour. The resin was filtered and the sodium acetate treatment was repeated. The acetate form of the resin was mixed with an equal volume of distilled water. 9 ml. of this suspension was pipetted into a 1 cm. x 25 cm. chromatographic tube. The walls were washed with 0.1 N acetic acid (about 10 ml.) until no more resin particles remained adhering to the walls of the tube. This left

a column of resin about 1 cm. x 6 cm. Next a layer of glass wool was packed in over the resin (about 1 cm. of glass wool).

The column can be regenerated after elution of neuraminic acid by adding 10 ml. of each of the following in the order given: 2.0 N NaOH, distilled water, 2.0 N sodium acetate and 0.1 N acetic acid.

The combined supernatants were passed through a column. The column was then washed twice with 5 ml. of distilled water. Then the N-acetylneuraminic acid was eluted from the resin with 9 ml. of acetate buffer (pH 4.6). The effluent was collected in a graduated tube and the volume was made up to 10 ml.

Then 2.0 ml. of eluate was used for the quantitative colour reaction. This was done by the resorcinol method (5).

Standard Curve for the Resorcinol Method -

N-acetylneuraminic acid (sialic acid) from Sigma was used (90% pure). Six mg. was made up to 100 ml., i.e., 54 μ g. per ml. Various volumes of the standard sialic acid solution were pipetted into tubes and made up to 2 ml. with water. Water blanks of 2 ml. were used. Two ml. resorcinol reagent was added into each tube, and the stoppered tubes were heated for 30 minutes in a boiling water bath. (The resorcinol reagent was made up by dissolving 0.2 g. of recrystallized resorcinol in 10 ml. water to which was added 80 ml. concentrated HCl and 0.25 ml. of 0.1 M CuSO_4 ; and finally water was added to 100 ml.) The tubes were removed from the water bath and cooled to room temperature. 5 ml. of isoamyl alcohol was added with vigorous shaking. The tubes were next chilled for 10 minutes in an ice-water bath, centrifuged for 5 minutes. The clear, blue coloured isoamyl alcohol layer was pipetted into cuvettes and read on the Beckman DU Spectrophotometer at 580 m μ . Each determination was done in duplicate.

Recovery of the standard sialic acid -

2.5 ml. (54 μ gm./ml.) of standard was diluted to 15 ml. with 0.1 N H_2SO_4 . This amount of the dilute standard was passed through each column to test its efficiency. This was then eluted as above, and an aliquot the eluate was used to determine the amount of standard sialic acid recovered from the column were found to be average (97.53%) in 3 such experiments.

Recoveries from gingival tissue of standard sialic acid are being undertaken the day of writing this report. This was dependent on acquiring gingival tissue samples large enough for duplicate determinations of neuraminic acid, and a residue for recovery estimations.

(5) Svennerholm, L., Acta Chemica Scand., (1958) 12, 547.

The Thiobarbituric Acid Method for Estimation of Sialic Acid

Because we did not, as a rule, obtain enough gingival tissue for the resorcinol method, it was necessary to use the thiobarbituric acid method after initial observations.

For this method we used 0.5 ml. of eluate which was obtained in the manner described above. 2.0 ml. water was used as a blank. To each tube was added 0.15 ml. of 0.2 M sodium periodate and the mixture was left at room temperature for 20 minutes. Following the addition of 1 ml. of 10% sodium arsenite, each tube was shaken by hand until a yellow colour disappeared. Following the addition of 3 ml. of 0.6% thiobarbituric acid (TBA) the tubes were heated for 15 minutes in a boiling water bath. The tubes were then cooled to room temperature and a 3 ml. aliquot was removed from each tube and added to 3 ml. cyclohexanone. After vigorous shaking the mixture was centrifuged. About 2.5 ml. of the pink supernatant phase was pipetted into cuvettes and readings were taken at once on the Beckman DU Spectrophotometer at 549 m μ .

A standard curve was established and recovery experiments were carried out as for the resorcinol method.

Nitrogen estimations on aliquots of each sample of hydrolyzed tissue were carried out by the micro Kjeldahl method. From the nitrogen values the amount of protein in the gingival tissue sample is calculated and values of sialic acid are reported as mg. per 100 mg. protein. This, in point of fact, in the case of sialic acid, gives a measure of the glycoprotein content of the tissue because the manner of hydrolysis acts on the linkage between protein and sialic acid and frees the latter. In this way, any discrepancies due to variations in tissue structure, water content, etc., are obviated.

Results

We have not yet proved that the sialic acid in gingival tissue is N-acetylneuraminic acid. This is to be done soon, in the manner we used previously with rat and human tissues. Consequently, we are reporting our results as mg. neuraminic acid or sialic acid. The results below do not include all our analyses, which total over 100. The control group of 14 samples is made up of material obtained from non-diseased gingiva. In addition, we give 3 other groups, without statistical analysis, or any attempt to classify the tissue according to disease states. This, of course, is to be done soon.

Table I

Neuraminic (Sialic) Acid Content of Normal
And Diseased Human Gingival Tissue

Group Number	Average Neuraminic Acid Content in mg.%/100 g. protein	Comments
I (14)	467.47	Normal tissue.
II (29)	329.94	Diseased, below control value. Lowest, in a chronic leukemia patient, was 223.26 mg./% protein.
III (30)	585.89	Diseased, above normal.
IV (10)	1,330.00	Diseased, very much elevated above normal.

(Numbers in brackets indicated number of patients)

Conclusions

1. A very wide range of values was obtained for sialic acid in gingival tissue.
2. Our preliminary observations indicate that there is an association with disease states of variations from the small "normal" group.
3. Our findings justify continuing with studies of other protein-bound carbohydrates in humans and in animals.

APPENDIX T

SUMMARY

PROGRESS REPORT ON RESEARCH PROJECT DA - 110

D. G. Woodside 1963

THE RELATION BETWEEN HUMAN MANDIBULAR GROWTH RATE AND FORM

- PHASE 1 (a) Assignment of a skeletal age rating to all children participating in the Burlington Orthodontic Research Project.
- (b) Re-assembly of the sample on a skeletal rather than a chronologic age basis.

A study to establish an objective method for recording the status of ossification and epiphyseal fusion in serial hand and wrist radiographs was completed. Four commonly used methods were assessed. No method provided a sufficiently accurate method to justify its use for assessment of the entire sample. Further methods will be assessed in an attempt to provide a reproduceable method of skeletal assessment.

- PHASE 2 (a) Determination of the maturation of the mandible in symmetry from measurements made from right and left oblique radiographs.
- (b) Determination of increments of mandibular growth and mandibular growth rate on a cross-section and serial basis.

A study to establish a method of assessing mandibular length from 45° rotated radiographs provided a reproduceable method for making such assessments.

Measurements of mandibular length have been completed for the entire male serial control sample and fifty members of the serial experimental group. Ages for each assessment have been converted into a decimal system. Data have been tabulated and growth rate and incremental growth curves constructed for each individual. Calculation of growth rates are in progress.

- PHASE 3 (a) Re-organization of the sample on the basis of retarded, normal or accelerated mandibular growth to determine if a relationship exists between growth rate and form.
- (b) Study of the relation between mandibular growth direction and facial type.

Measurements of mandibular form have been completed for the entire male serial control sample and fifty members of the serial experimental group.

A study to establish a method for assessing facial type and the incidence of the various facial types in a typical population of eight year old children has been completed.

W.V. Youdelis

Summary of Progress Report - 1963

University of Alberta

DA-95: Fundamentals of amalgam metallurgy and development of dental amalgams

During the past year, the scope of the research has been broadened to include the general field of dental materials. This report is made up of four separate sections, each of which describes a particular detailed study.

1. Production of metal alloy powder by atomization process.

The silver-copper eutectic dispersion alloy has been successfully atomized to a size range below 400 mesh and in sufficient quantities for testing purposes.

2. Further tests on amalgam were carried out using the atomized, spherical-shaped, dispersion powder. The compressive strength of the dispersion strengthened amalgam (30 weight percent dispersion powder, -500 mesh) reached levels of 70,000 p.s.i. compared with approximately 50,000 p.s.i. for commercial amalgam.

3. & 4. Effect of gallium and indium additions to amalgam. Preliminary results show considerable improvement in the compressive strength of the amalgam for relatively small additions of gallium and indium. The wetting affinity of mercury is increased appreciably.

Also, a preliminary study of the effect of germanium on the hardness of dental gold alloy has been undertaken, however, no conclusive results are yet available.

Members of the Associate Committee on Dental Research

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* 1. Dr. J.P. Lussier, <u>Chairman</u> Faculty of Dental <u>Surgery</u> , University of Montreal, Montreal, Quebec.	31 March 1964
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* 5. Dr. L. E. Francis, Dept. of Clinical Dentistry, McGill University, Montreal, Quebec	31 March 1965
6. Dr. R.M. Grainger, Division of Dental Research, University of Toronto, Toronto, Ontario.	31 March 1965
7. Dr. A. M. Hunt, Faculty of Dentistry, University of Toronto, Toronto, Ontario.	31 March 1966
* 8. Dr. I. Kleinberg, Dept. of Biochemistry, University of Manitoba, Winnipeg, Manitoba	31 March 1965

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Vancouver, British Columbia 31 March 1966
- * 10. Dr. G. Nikiforuk,
Division of Dental Research
University of Toronto,
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NATIONAL RESEARCH COUNCIL OF CANADA

PROCEEDINGS
OF THE
SIXTEENTH MEETING
OF THE
EXECUTIVE
ASSOCIATE COMMITTEE ON
DENTAL RESEARCH

OTTAWA

30 OCTOBER 1964

ASSOCIATE COMMITTEE ON DENTAL RESEARCH

EXECUTIVE

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NATIONAL RESEARCH COUNCIL

Proceedings

of the

Sixteenth Meeting*

of the

EXECUTIVE

ASSOCIATE COMMITTEE ON DENTAL RESEARCH

30 October 1964.

The meeting convened in the Council Chamber of the National Research Council, Ottawa, at 9:30 a.m., 30 October 1964.

1. Attendance: Dr. J.-P. Lussier, Chairman
Dr. L. E. Francis
Dr. A. G. Gornall
Dr. R. M. Grainger
Dr. I. Kleinberg
Mrs. J. A. Van Laer, Secretary

The Chairman welcomed Dr. R. M. Grainger as a new member to the Executive, replacing Dr. G. Nikiforuk. It was noted that Dr. Nikiforuk had left Canada, and therefore would be unable to continue as a member of the Associate Committee on Dental Research.

2. Secretary of the Committee

The Chairman noted the appointment of Mrs. J. A. Van Laer as Secretary to the Committee, replacing Mr. A.D. Armstrong.

3. Executive Action Since March

The Chairman drew attention to the following Executive Action taken since the last meeting:

* The Fifteenth Meeting of the Executive was held in March 1957. Meetings of the Executive held in the years following were informally conducted prior to main Committee meetings, and resulted in no written Proceedings.

- Third Canadian Conference on Dental Research

The Council approved a grant of \$2,500 towards the Third Canadian Conference on Dental Research, held 5-7 October 1964 at Ste-Marguerite, Quebec.

- Fellowships

Taichman, N.S. Declined Fellowship to study in the Dept. of Pathology, Banting Institute, University of Toronto, under Dr. H.Z. Movat, in order to accept an award from the J.B. Bickell Foundation

Weinstock, A. Requested Supplement (\$300) to Fellowship at Harvard; to study oral pathology at the Armed Forces Institute of Pathology in Washington during summer 1964.
Supplement not Granted

- Associateship

Goldner, M. Amount of Award Reduced; University of Toronto recommended a salary of \$10,500, as opposed to that of \$11,500 suggested by the Associate Committee on Dental Research.

- Grant

Cleall, J.F.	<u>New Grant Awarded</u>
Orthodontics	DA-131: Growth and growth deviations
Manitoba	of the cranio-facial skeleton.
	Awarded \$1,290

4. Meeting with Council Officers

The Chairman then reported on a meeting he had attended on 4 September with Dr. Leo Marion, Dr. J. B. Marshall, and Mr. F. L. W. McKim, to consider the problems confronting the Associate Committee during the past few years. At that time, the Chairman had expressed concern that the policies of the National Research Council did not always conform to the pattern established by the Associate Committee on Dental Research. The Committee felt, therefore, that other means of furthering dental research in Canada should be considered.

a) Financial Requirements for Dental Research

The financial situation and difficulties caused by the inadequacy of funds to support dental research in Canada had been discussed as well at the 4 September meeting, and an estimate of requirements for 1965-66 presented.

b) Intercommittee Representation

The Executive then considered the proposal for intercommittee representation. It had been suggested at the 4 September meeting that, rather than members of the Associate Committee attending the meeting of the Grant Selection Committees in March, specific representatives of the Council be invited to sit in on the meeting of the Associate Committee. These representatives, with their overall knowledge of Council policies, could make suggestions or advise concerning methods and procedures of the Associate Committee whenever necessary.

The Executive agreed to act upon this proposal.

5. Representation at Meeting of Conveners

It was noted, however, that the Associate Committee might better understand Council financial policies, and would be able more effectively to make its own need understood if the Chairman were to attend the annual meeting of Grant Selection Committee Conveners at which the allocation of funds is determined.

The Secretary was requested to make further arrangements to this end.

6. Support of Clinical Research

The Executive then reviewed Motion 22 of the Proceedings of the Thirtieth Meeting of the Associate Committee on Dental Research, concerning the support of clinical research by the Committee.

After discussion, it was agreed that the Associate Committee should support only clinical research of superior quality, comparable in calibre to the basic research now supported by the Committee.

It was decided, therefore, that the Chairman would write a letter to the Deans of Canadian Dental Schools informing them that the Associate Committee on Dental Research encourages applications for the support of clinical research, but pointing out that other sources, such as the Department of National Health and Welfare, to an even greater extent, finance projects in clinical research.

In addition, this letter would request that the N.R.C. instructions pamphlet "Awards to University Staff" be examined carefully before the submission of applications for such research, to ensure that requests were for the type of research normally supported by the Council.

7. Committee to Evaluate the Performance of an Associate

The Executive next considered procedures for evaluating the performance of a Dental Research Associate prior to renewal of his award after the initial three-year tenure. Following discussion, it was decided:

- a) that a subcommittee of the Associate Committee be formed to determine criteria for evaluating the performance of an Associate. The following members of the Associate Committee would be asked to serve on this subcommittee, to be known as the Rules Committee:

Dr. C. P. Leblond, Chairman
Dr. J.-P. Lussier
Dr. A. J. Rhodes

- b) that the Executive be empowered to select referees to review the work of an Associate. These referees would be chosen according to their familiarity with the type of work done by the Associate in question, and their ability to appreciate the relationship between the Associate's work and dental research.

8. Associateship of Dr. E. T. Pritchard

The Executive recommended that the following persons be asked to serve as referees to review the work of Dr. E. T. Pritchard at the University of Manitoba, whose Associateship comes up for renewal in March 1965:

Dr. R. J. Rossiter
Dept. of Biochemistry,
University of Western Ontario

Dr. C. C. Lucas
Best Institute
University of Toronto

Dr. J.-P. Lussier
Faculty of Dental Surgery,
University of Montreal

9. Rating Scale for Referees - Applications for Grants

The present referee report form used by the Associate Committee was discussed at length by the Executive and completely revised, with particular reference to page 4 of the "Application for Grant in Aid of Research (revised 1-3-64)". The new form would be known as the Reviewer's Assessment Form.

10. Method of Reviewing Applications for Grants

The present method of reviewing grant applications was also examined and revised.

The Executive drew up a list of subdivisions of dental research and assigned members of the Associate Committee to each field. Members selected would be known as the Referee Panel.

The Chairman agreed to divide the grant applications, when received, into these categories, and to distribute them to the Panel members respectively concerned. The Panel would then assign them to referees specifically informed in the field of research of the applicant assigned. Referees would not necessarily be members of the Committee.

Members of the Referee Panel and the field for which each is responsible are listed below:

Anatomy	-	Dr. C. P. Leblond Dr. L. F. Bélanger
Biochemistry	-	Dr. A. G. Gornall Dr. A. M. Hunt Dr. I. Kleinberg

Physiology	-	Dr. S. Wah Leung Dr. J.-P. Lussier
Bacteriology	-	Dr. R. V. Blackmore Dr. J. C. Wilt
Pharmacology	-	Dr. L. E. Francis
Pathology	-	Dr. L. E. Francis
Growth and Development	-	Dr. R. M. Grainger
Dental Materials	-	Dr. I. Kleinberg
Clinical Dentistry-		Dr. S. Wah Leung

11. Recommendations 159 and 177 of the Hall Report

159: That the Health Sciences Research Council endeavour to expand in Canada the amount of research on dental disease and prevention, both by conducting intra-mural research and by increasing its grants to the universities;

177: That the Medical Research Council be broadened by appropriate legislation to include all fields of health research, and renamed the Health Sciences Research Council; as so reconstituted it be recognized by the Government of Canada as its principal advisor in the planning and support of health research and the allocation of research funds; its services be available to provincial governments, voluntary health associations, and universities; and further, that in the proposed Act for expansion of the Council, there be provision for the appointment of additional outstanding persons from the health and other professions. An outstanding "layman", not connected with any particular health services programme or agency, should be appointed Chairman. This does in no way imply any criticism of the structure of the present Medical Research Council or of its distinguished Chairman, but in the expanding role of the Council recommended here, embracing other health fields, we believe that a neutral person, rather than a member of one of the health professions, would be desirable as Chairman as and when a vacancy occurs.

12. Report on Research Support Through the Associate Committee

The Executive discussed the above two recommendations in conjunction with a report presented by Dr. R.M. Grainger concerning "Research Support Through the Associate Committee on Dental Research of the National Research Council".

It was decided that Dr. Grainger's Report should be prepared for publication and brought to the attention of the National Research Council.

13. Reassessment of the Associate Committee

The Chairman drew attention to the fact that the present activities and aims of the Associate Committee on Dental Research are not in line with N.R.C. policy regarding Associate Committees, and that a revaluation of the terms of reference of the Committee was needed. He expressed the opinion that the Associate Committee on Dental Research has closer affiliation with the Medical Research Council, since it is concerned, like M.R.C., primarily with health.

It was therefore decided that the Committee would work for better recognition by the National Research Council, or a possible favorable association with the Medical Research Council.

14. Future of the Associate Committee

The Executive agreed, furthermore, that the Associate Committee on Dental Research should in future become an autonomous part of the Health Sciences Research Council recommended by the Hall Commission.

To this end, they determined to obtain all information possible; including recommendations from Committee Chairmen of the Third Canadian Conference on Dental Research, opinions of the Canadian Dental Association, the Department of National Health and Welfare, Public Health Departments, and the dental professions; as well as the views of N.R.C. authorities. A Report would then be prepared to be presented to the Associate Committee at its meeting in March 1965, containing recommendations as to the future structure of the Health Sciences Research Council incorporating a Dental Research Division as an autonomous unit.

15. Third Canadian Conference on Dental Research

The Chairman then reported on the success of the Third Canadian Conference on Dental Research, and informed the Executive that a full report on the Conference would be presented to the National Research Council.

16. New Business

a) Application for Major Equipment Grant

One new application for a Major Equipment Grant was considered, as follows:

Kraintz, Leon	Requested \$7,500 for a
Oral Biology	Technicon Autoanalyzer for
Br. Columbia	Fluorometric Determinations.

Due to lack of funds, the Executive agreed to recommend to Council that Dr. Kraintz's application be rejected at this time, but that he be requested to submit this, or a revised application again in March 1965.

b) Application for Senior Research Fellowship

One application for a Senior Research Fellowship was also considered by the Executive, as follows:

Bingham, R.H.	To spend 8 months in London,
Oral Diagnosis	England pursuing the study
Dalhousie	of methods used in diagnosing
	lesions of the soft oral tis-
	sues; and in investigating
	the diagnosis and treatment
	of mandibular joint disorders,
	at various institutions; also
	to study the operations of the
	dental clinics of these insti-
	tutions.

The Executive agreed to recommend this application as low priority, since Dr. Bingham's work is basically clinical, and the activities of his sabbatical leave would do little to advance dental research in Canada.

17. Improvement of Dental Training

Dr. A. G. Gornall drew attention to the need in Canadian universities for an Honours Science and Dentistry course to train future dental research workers more thoroughly in the basic sciences.

The Executive agreed that this would provide a great furtherance to dental research in Canada, and asked Dr. Gornall to prepare a recommendation to this effect for presentation to the Associate Committee at its meeting in March 1965.

18. Annual Fall Meeting of the Executive

It was agreed that the resumption of fall meetings of the Executive of the Associate Committee would enable Committee business to be conducted more efficiently.

The meeting adjourned at 3:45 p.m.

Distribution

(i) to members of the
Associate Committee on Dental Research

- | | |
|--------------------------------|------------------------------------|
| 1. Dr. J.-P. Lussier, Chairman | |
| 2. Brigadier K. M. Baird | 11. Dr. R. M. Grainger |
| 3. Dr. B. G. Ballard | 12. Dr. A. M. Hunt |
| 4. Dr. L. F. Belanger | 13. Dr. I. Kleinberg |
| 5. Dr. R. V. Blackmore | 14. Dr. C. P. Leblond |
| 6. Dr. R. C. Connor | 15. Dr. S. Wah Leung |
| 7. Dr. R. F. Farquharson | 16. Dr. A. J. Rhodes |
| 8. Dr. L. E. Francis | 17. Dr. D. M. Tanner |
| 9. Dr. F. W. Fyfe | 18. Dr. J. C. Wilt |
| 10. Dr. A. G. Gornall | 19. Mrs. J. A. Van Laer, Secretary |

(ii) to other persons

20 - 24 Deans of Dental Schools (who are not members of the Committee)

- | | |
|---------------|-------------------|
| 20. Alberta | Dr. H. R. MacLean |
| 21. Dalhousie | Dr. J. D. McLean |
| 22. Manitoba | Dr. J. W. Neilson |
| 23. McGill | Dr. J. McCutcheon |
| 24. Toronto | Dr. R. G. Ellis |

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NATIONAL RESEARCH COUNCIL OF CANADA

PROCEEDINGS
OF THE
THIRTY-FIRST MEETING
OF THE
ASSOCIATE COMMITTEE ON
DENTAL RESEARCH

OTTAWA

10-11 MARCH 1965

ASSOCIATE COMMITTEE ON DENTAL RESEARCH

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NATIONAL RESEARCH COUNCIL

2. Chairman's Report Proceedings

The Chairman of the
Thirty-First Meeting
of the

ASSOCIATE COMMITTEE ON DENTAL RESEARCH

10 - 11 March, 1965

The first session convened in the Council Chamber of the National Research Council, 100 Sussex Drive, Ottawa, at 9:30 a.m., 10 March 1965.

1. Attendance

Dr. J.-P. Lussier, Chairman
Dr. L. F. Bélanger
Dr. R. V. Blackmore
Dr. R. A. Connor
Dr. R. F. Farquharson
Dr. L. E. Francis
Dr. F. W. Fyfe
Dr. A. G. Gornall
Dr. R. M. Grainger
Dr. A. M. Hunt
Dr. I. Kleinberg
Dr. C. P. Leblond
Colonel A. C. Leman
Dr. S. Wah Leung
Dr. K. J. Paynter
Dr. D. M. Tanner
Dr. J. C. Wilt
Mrs. J. A. Van Laer, Secretary

Regrets at their inability to attend were received from:

Dr. A. J. Rhodes
Brigadier K. M. Baird (Dept. of National Defence)

The Chairman extended a welcome to the new member of the Committee, and to the guests, introducing Colonel A. C. Leman, who attended in place of Brigadier K. M. Baird; and Dr. K. J. Paynter, Chairman of the Council on Dental Research of the Canadian Dental Association.

2. Chairman's Remarks

The Chairman introduced his remarks as follows:
"This should not be taken as a Throne Speech. Yet these words were chosen with the intent of bridging the actions you have taken last year with those you may have to take in the coming days. Since we last met, some twelve months ago, events of importance to us have taken place, some on account of our own decisions, others independently of them. It would be opportune to have a short review before we begin our deliberations.

i. The Publication of the Hall Commission's Report: June, 1964

Among other responsibilities, the Commission had to face the difficult task of finding means to meet a large magnitude of needs for dental care. Obviously, the most promising means in the present situation is prevention. Preventive programs, to be successful, are largely dependent on active research in basic and applied fields. In the first volume of the Report, recommendations were expressed in broad terms, leaving to the suggested 'Council on Health Research' the duty of integrating dental research in a full health research program.

At this moment, it is left to the dental research workers to demonstrate their ability to come up with the most promising proposals for the development of dental research, simultaneously with, and not independently of, other research fields.

ii. The Third Canadian Conference on Dental Research

This Conference, sponsored partly by the National Research Council and partly by Proctor and Gamble Company, was a timely event to initiate free discussion on the future of dental research. Research workers and dental school administrators congregated at Ste Marguerite, Quebec, 5 - 8 October 1964. Research communications were presented at two sessions. The participants, grouped as a workshop, spent three sessions discussing the development of dental research in Canada; at the last session, statements were submitted and recommendations approved. It is expected that the pro-

ceedings of this workshop will soon be published in the Journal of the Canadian Dental Association. The Conference was rated as very successful, and it is anticipated that this organization will soon become a yearly affair.

iii. Meeting of the Executive: October 1964

The Executive of the Associate Committee on Dental Research met last 30 October, and as indicated by the variety of questions dealt with on this occasion, it was a profitable session. It is expected that the Executive will, from now on, have a meeting each fall. Many things can thus be accomplished which otherwise would have to await consideration at the annual spring meeting of the Committee.

iv. Appointment of the Chairman of the Associate Committee on Dental Research to the Department of National Health and Welfare Advisory Committee

By extension of courtesy from the Dental Division of the Department of National Health and Welfare, the Chairman of the National Research Council Associate Committee on Dental Research was invited last January to join as a member of the newly formed Advisory Committee on Dentistry.

The reciprocity of liaison between the Department of National Health and Welfare and the Associate Committee on Dental Research is highly commendable. The better our communications, the more effective our coordination. As both groups are striving towards the same objective, i.e. to bring as large as possible a support to dental research in Canada, it is obvious that they should work in constant harmony of means and efforts. Later during the meeting, we shall have an opportunity to learn more about the Dental Division of the Department of National Health and Welfare when we hear Dr. R.A. Connor, the Director.

v. Interdepartmental Medical Research Coordinating Group

Again this year, the Chairman of the Associate Committee on Dental Research was invited to attend the Inter-

Medical Research Coordinating Group meeting. It is always a rewarding experience to meet with the top administrators of medical research in this country. Yet it may be frustrating at times to be made aware of the resources available for medical research (for 1965-66, over 22 million dollars to meet requests for 30 million), while dental research must put up with less favorable conditions.

vi. Visits to Institutions

In a more or less official capacity, the Associate Committee on Dental Research Chairman was able to gather valuable information about the conditions under which dental research is carried out in Canada and the United States.

Nowadays, to be a respected dean, one must travel freely. Being personally willing to be worthy of consideration from this standpoint, I do not lose too many opportunities to get around. Incidentally, I was given the chance during the past year;

- a) to visit the University of Alberta Dental Faculty and its dental research quarters, last June,
- b) to attend the International Dental Federation, Council of Research Meeting, last November in San Francisco,
- c) to visit the University of Manitoba, Faculty of Dentistry, and particularly the Department of Oral Biology, last February,
- d) to visit some American dental schools, namely the University of Indiana, Harvard University and the Forsyth Institute; where graduates of the Canadian Dental Faculties are following graduate programs, some of them under NRC sponsorship. I had once again the opportunity to be convinced further of the strong potentialities existing in the Canadian schools, the quality of the research done therein, and the necessity of bringing this endeavor to a maximum

yield. The main difference between the American and the Canadian institutions is mainly quantitative; i.e., the availability of staff and space, and the magnitude of material aids. Fortunately, though, there is no difference in the creativity, the incentive and the determination of our workers, when compared to our neighbors. If a difference does exist, I would rather say it favors my fellow-citizens.

- e) last, but not least, I was granted the privilege of meeting with representatives of the National Research Council Executive: Vice-President (Scientific), L. Marion; Director, F. L. W. McKim; and Awards Officer, J. B. Marshall, early last September. How effective was my plea for more liberal support will be found as we progress in the Agenda."

3. Minutes of the Thirtieth Meeting

The Minutes of the Thirtieth Meeting had been distributed to all members of the Committee.

It was moved by Dr. A. G. Gornall and seconded by Dr. L. E. Francis, that the Minutes be accepted as read and approved.

CARRIED

4. Minutes of the Sixteenth Meeting of the Executive

The Chairman reported briefly on the Sixteenth formal Meeting of the Executive, the Minutes of which had been distributed to all members of the Committee.

It was moved by Dr. R. M. Grainger and seconded by Dr. I. Kleinberg, that these Minutes be accepted as read and approved.

CARRIED

5. Executive Action Since the Sixteenth Meeting of the Executive

The Chairman drew attention to the following action taken by the Management Committee of the Council since 30 October 1964.

Jordan, R. E.	<u>Operating Grant Reduced</u>
Dentistry,	DA-106: Human primary molar
Dalhousie	development
	Original Grant \$1,000.00
	Decrease 985.60
	Net Grant 14.40

Accepted appointment at the University of Pittsburgh

Kropp, B. N.	<u>Operating Grants Reduced</u>
Histology &	DA-91: 1961-62
Embryology,	The influence of fluoride on
Queen's	differentiation of calcified
	structures and on teratogenesis
	Original Grant \$4,385.00
	Decrease 3,674.05
	Net Grant 711.95

DA-103: 1962-63

An investigation of the effect of salivary gland extract, saliva and sialagogues on growth of teeth

Original Grant	\$1,750.00
Decrease	1,141.80
Net Grant	608.20

6. Financial Status of the Committee

The Secretary informed the Committee that:

- a) \$215,000 would be available during 1965-66 for grants in dental research. Of this amount,
 - \$32,500 is committed for Term Grants,
 - \$14,000 for Summer Undergraduate Assistantships;leaving \$168,500 for new Operating and Major Equipment Grants;
- b) \$3,200 would be available for Committee Travel and Administration expenses.

Attention was drawn to the fact that Fellowships, Scholarships and Associateships are paid from a general NRC fund for the payment of all types of scholarships; therefore, the Committee's allotment is calculated in terms of number of awards rather than amount, for such categories.

For 1965-66, the Committee would be allowed to award a maximum of 16 Fellowships. No new applications for Scholarships or Associateships were received.

7. Informal Meeting of the Executive

The Chairman informed the Committee that the members of the Executive (Dr. J.-P. Lussier, Dr. L.E. Francis, Dr. A. G. Gornall, Dr. R. M. Grainger and Dr. I. Kleinberg) had met on 9 March 1965 at 9:30 a.m. A brief report had been prepared outlining the suggested recommendations for Fellowships, Scholarships, Associateships and Grants, reached in the Executive's preliminary consideration.

8. Applications for Fellowships

A. 1965-66 Requests

Twenty applications for Graduate Dental Research Fellowships tenable during 1965-66 were considered: seven for renewal of current Fellowships, and nine for a first award. Action was taken as follows:

<u>NAME</u>	<u>TO STUDY AT</u>	<u>AMOUNT</u> <u>RECOMMENDED</u>
		\$
Ambrozaitis, J.	University of Montreal Dept. of Bacteriology, under Dr. G. Nogradý	4,700
Bell, P. A.	University of Toronto Dept. of Oral Pathology under Professors H.G. Poyton and H.A. Hunter	Nil

Binnie, W. H.	Indiana University, Dept. of Oral Diagnosis and Oral Medicine, under Dr. D.F. Mitchell	4,000
Biswas, S. D.	University of Manitoba, Dept. of Oral Biology, under Dr. I. Kleinberg	Nil
Boyko, J.	University of Manitoba Dept. of Oral Biology, under Dr. C.M. Dowse	Nil
Freedman, H. L.	University of Toronto Banting Institute, Dept. of Pathology, under Dr. H. Z. Movat	4,700
Golub, L. M.	Harvard School of Dental Medicine, Dept. of Oral Biology, under Dr. P. Goldhaber	4,500
Haselton, R. D.	University of Rochester, Dept. of Anatomy under Dr. E. Johansen	Declined
Kaufman, H. W.	University of Manitoba Dept. of Oral Biology under Dr. I. Kleinberg	4,800
Lawlor, R.	University of Montreal Dept. of Bacteriology under Dr. S. Sonea	3,500
Lebuis, J.-J.	University of Montreal Dept. of Anatomy, under Dr. E. Sandborn; Villejuif, France, under Dr. W. Bernhardt	Nil
Nichol, N. E.	University of Manitoba Dept. of Oral Biology, under Dr. E. T. Pritchard	4,000

Sandham, H. J.	University of Manitoba, Dept. of Oral Biology, under Dr. I. Kleinberg	5,600
Silverman, G.	University of Manitoba, Dept. of Oral Biology, under Dr. I. Kleinberg	3,500
Swanson, W. D.	University of Michigan, Dept. of Dental Materials, under Prof. F. A. Peyton	Declined
Thompson, G. W.	University of Toronto, Faculty of Dentistry, under Dr. R. M. Grainger	3,500
Weinstock, A.	Harvard School of Dental Medicine, Dept. of Histopathology, under Dr. P. Goldhaber	4,500
Weinstock, M.	Forsyth Dental Center, Dept. of Orthodontics, under Dr. J. T. Irving	3,500
Young, C. L.	University of Toronto, Faculty of Dentistry, under Dr. H. G. Poyton	3,500
Zebrowski, E. J.	University of Manitoba, Dept. of Oral Biology, under Dr. E.T. Pritchard	Nil

It was moved by Dr. L. E. Francis and seconded by Dr. L. F. Bélanger, that payment of these grants be made as outlined above.

CARRIED

In determining the amount of each award, the Committee agreed that additional allowances be granted to:

- a) Fellows with children (\$500 for the first and \$200 for each additional child). This allowance had been waived in most cases during 1964-65 due to lack of funds.

- b) Applicants studying in the United States, because of the difference in the dollar value.
- c) Applicants with additional degrees (such as M.Sc.); considered specifically in connection with the number of years spent in school.

B. Support of Ph.D. Research Workers

With regard to the applications of Dr. S.D. Biswas and Dr. E. J. Zebrowski, the Committee discussed the question of whether support for research workers with a Ph.D. should come within the terms of reference for Graduate Dental Fellowships.

The Committee examined the NRC awards offered for Postdoctorate Fellowships in Canada and overseas, and consulted with Dr. J. B. Marshall, Awards Officer, regarding the possibility of applicants in dentistry qualifying for these.

Although Dr. Marshall confirmed the eligibility of candidates in dental research for NRC Fellowships, the Committee was uncertain as to the advantages of these particular awards.

After further discussion, it was agreed that the Executive should look carefully into all possibilities, in order to decide what is the most effective means of encouraging dental research at the Ph.D. level.

This investigation would include exploring with authorities of the National Research Council the possibility of establishing a program of postdoctorate awards in dentistry sponsored by the Associate Committee.

For 1965-66 however, it was agreed that until further information was obtained, the Ph.D. research workers, Dr. S. D. Biswas and Dr. E. J. Zebrowski should be supported under the operating grants of their supervisors, Dr. I. Kleinberg and Dr. E. T. Pritchard respectively. Money would be allocated from the grants budget for this purpose.

C. Support of Fellows in Clinical Dentistry

The Committee then considered at length the policies governing the terms of reference for Fellowships with regard to support of candidates from the clinical fields of dentistry.

With the information available, it was felt that no decision could be reached at present as to whether the Committee should expand its terms of reference to include support of students doing clinical work, or whether Fellowships should be restricted to candidates devoting their time primarily to research training.

Therefore, it was moved by Dr. I. Kleinberg and seconded by Dr. J. C. Wilt, that a special subcommittee be appointed to study the whole problem of Dental Fellowships from the point of view of what is best for the future of dentistry in Canada, and that this subcommittee prepare a report to be presented to the Executive when it meets in the fall of 1965.

CARRIED

Dr. L. E. Francis then moved, and Dr. I. Kleinberg seconded, that the members of this subcommittee be as follows:

Dr. R. M. Grainger, Chairman
Dr. K. J. Paynter
Dr. A. M. Hunt

CARRIED

9. Applications for Scholarships

The salary of the incumbent holding the Dental Scholarship tenable during 1964-67 was considered. Action was taken as follows:

	1964-65	1965-66
		Recommended Approved
R. C. Burgess	\$8,500*	\$8,800* \$8,800*
Faculty of Dentistry		
University of Toronto		

*Plus payment of Scholar's share of cost of pension and other benefits in which university staff members normally participate.

10. Applications for Associateships

In connection with the application of Dr. E. T. Pritchard for renewal of his Associateship, the Chairman drew attention to:

- a) the outline of "Instructions for the Use of Investigators of Research Associates";

With regard to these "Instructions", Dr. R.F. Farquharson suggested an amendment under "Research Work", requesting an outline, from the Associate in question, of any new knowledge which had emanated from his work.

It was moved by Dr. L. F. Bélanger and seconded by Dr. I. Kleinberg, that the "Instructions for the Use of Investigators of Research Associates" be accepted as amended.

CARRIED

- b) the Report of the Committee of Enquiry appointed to evaluate the performance of Dr. E. T. Pritchard;

The Chairman briefly summarized the Report of the Committee of Enquiry investigating Dr. E. T. Pritchard, pointing out that Dr. Pritchard had fulfilled all the requirements specified for a Dental Research Associate, and noting that the only deficiency in his case was a lack of adequate laboratory space, a situation which is to be rectified as soon as possible.

The Committee of Enquiry therefore had recommended renewal of Dr. Pritchard's Associateship for a further tenure of 5 years.

It was thereupon moved by Dr. A. G. Gornall and seconded by Dr. S. Wah Leung, that the Report of the Committee of Enquiry be accepted, and that

Dr. Pritchard's Associateship be renewed for a further five years.

CARRIED

c) Dr. Pritchard's Report on the work done during tenure of his Associateship;

The Committee of Enquiry had considered this in preparing their recommendation. The Associate Committee examined it briefly, and noted that it confirmed the conclusions of the Committee of Enquiry regarding limitations of space.

11. Salary of the Associates

The Committee then considered the salary of the two incumbents holding Dental Research Associateships; action was taken as follows:

	1964-65	1965-66	
		Recommended	Approved
Dr. M. Goldner	\$10,500*	\$11,500*	\$11,500*
Faculty of Dentistry			
University of Toronto			
Associateship tenable 1964-1967			
Dr. E. T. Pritchard	\$10,300*	\$11,300*	\$11,300*
Faculty of Dentistry			
University of Manitoba			
Associateship tenable 1965-1970			

*Plus payment of Associate's share of cost of pension and other benefits in which university staff members normally participate.

12. Applications for Grants

a) Term Grants

The Chairman noted that funds were already committed in the coming year for Term Grants as follows:

Name	Department	University	Project Title	Requested \$	Recommended \$
Copp, D. H.	Physiology,	Br. Col.	Phosphorus and calcium deficiency: effects on bones and teeth DT-59	7,000 (Term - 2nd Inst.)	7,000
Francis, L.E.	Pharmacol. & Therap.,	McGill	Investigation of a gingival histamine and serotonin antagonist DT-76	5,000 (Term - 3rd Inst.)	5,000
Hunt, A. M.	Dentistry,	Toronto	Measurement of strontium ⁹⁰ and carbon ¹⁴ in primary teeth of Canadian children DT-82	8,000 (Term - 3rd Inst.)	8,000
Kleinberg, I.	Oral Biol.,	Manitoba	1. Metabolism of dental plaque 2. The effect of refinement of dietary carbohydrate on dental caries	12,500 (Term - 2nd Inst.)	12,500 6,000 (supp.)*

*\$6,000 was added to the grant of Dr. I. Kleinberg as a one-year supplement, to provide support for Dr. S. D. Biswas during 1965-66.

Thirty-two applications for Operating Grants, seven for grants to support Summer Undergraduate Assistants, and two for Major Equipment Grants, were then considered. Each application had been sent to Reviewers designated by the Referee Panel prior to the meeting, and copies of the Reviewers' Assessments were made available at the meeting. Also at hand was a list of recommendations prepared by the Executive during their preliminary review.

With respect to Term Grants, the Committee agreed that established investigators required adequate support, and confirmation of continuing increases in future years.

It was therefore decided to award three large new Term Grants in 1965-66, which would be increased by approximately 10% each year during tenure of the grants. It was felt that this policy would assure senior research workers of support in proportion to their needs as they expanded, and would also discourage supplemental requests.

Awards were therefore recommended as follows:

Name	Department	University	Project Title	Requested \$	Recommended \$
Dowse, C. M.	Oral Biol.,	Manitoba	Metabolism of bone DT-81	17,175	12,000 (Term-1st Inst. 2nd Inst. - 14,000 3rd Inst. - 16,000)
Leblond, C. P.	Anatomy,	McGill	Formation of matrix of enamel and dentin DT-23	10,450	9,000 (Term-1st Inst. 2nd Inst.- 10,000 3rd Inst. 11,000)
Pritchard, E.T.	Oral Biol.,	Manitoba	Sulfolipids of rat tissues DT-109	14,300	11,000 (Term-1st Inst. + 6,000supp.* 2nd Inst.- 14,000 3rd Inst.- 17,000)

*\$6,000 was added to the grant of Dr. E. T. Pritchard as a one-year supplement, to provide support for Dr. E.J. Zebrowski during 1965-66.

b) Operating Grants

The following Annual Grants were recommended as follows:

Name Department <u>University</u>	<u>Project Title</u>	<u>Requested</u> \$	<u>Recommended</u> \$
Arora, B.K. Oral Surg., Toronto	Effect of denervation of muscles of mastication on growth of mandible of pigs DA-132	1,000	800
Blackmore, R.V. Dental Res., Alberta (Ed.) Manitoba	a) Characterization of a viral inhibitory factor produced when animal cells in culture are exposed to <u>Herpesvirus hominis</u> b) The phenomenon of immunologic tolerance as related to experimental herpetic infection of rabbits DA-102	7,134	7,000
Burgess, R.C. Dental Res., Toronto	Further studies on proteins in developing enamel DA-130	7,450	6,950
Cleall, J.F. Oral Biol., Manitoba	Growth and growth deviations of the craniofacial skeleton DA-131	4,620	3,700
Cleall, J.F. & Kawata, T. Oral Biol., Manitoba	Biochemical changes induced by orthodontic stimuli	6,800	Nil
Das, A.K. Dentistry, Dalhousie	1. Studies on the mechanism of growth and repair of the mandibular condyle and temporomandibular joint in the rat. DA-133	6,350	2,000

<u>Name</u> <u>Department</u> <u>University</u>	<u>Project Title</u>	<u>Requested</u> \$	<u>Recommended</u> \$
Das, A.K. Dentistry, Dalhousie	2.Experimental pro- duction of leukoplakia in hamster by beetle- nut, tobacco and se- veral other ingredients used for chewing.	4,650	Nil
Dawes, C. Oral Biol., Manitoba	a) Factors influencing the secretion and preci- pitation of salivary proteins b) The relation between plaque fluoride content and the concentration of fluoride in drinking water DA-122	12,390	9,900
Edmund, A.G. Vert.Palaeont. Roy.Ont.Mus. Toronto	Dynamics of tooth re- placement processes in modern reptiles DA-104	600	500
Goldner, M. Dental Res., Toronto	Studies on antibiotic- inactivating enzymes of bacteria: Pencillinase and cephalosporinase DA-129	13,600	10,000
Grainger, R.M. Hygiene & Dent. Toronto	Statistical consulta- tion and analysis unit DA-123	6,059	5,000
Hamilton, I.R. Oral Biol., Manitoba	Anaerobic carbohydrate metabolism by strepto- coccus salivarius DA-124	12,540	10,000

<u>Name</u> <u>Department</u> <u>University</u>	<u>Project Title</u>	<u>Requested</u> \$	<u>Recommended</u> \$
Johnson, E.W. Mech. Eng., Alberta (Ed.)	Three-dimensional photoelastic analysis of stress concentration in operatively involved human teeth DA-134	4,250	3,200
Johnson, R.H. Oral Path.& Diagnosis, McGill	The role of the mast cell in the oral cavity DA-135	2,650	2,700
Johnston, M.C. Dental Res., Toronto	Studies on the embryo- logical development of the face DA-125	7,695	6,500
Kasloff, Z. Restor. Dent., Manitoba	Metallographic studies of soldered joints and cast gold parent metals DA-108	8,195	6,600
Khairat, O. Bacteriol.& Immunol., Manitoba	1. The mechanism that allows growth of dental anaerobic bacteria, aerobically in the mouth and on the gums. 2. Studies on some anaerobes (fusiformis) isolated from the circulating blood after teeth extraction DA-101	4,354	3,400
Lague, J.-G.& Lussier, J.-P. Dental Surg., Montreal	Etude du métabolisme du citrate dans les tissus minéralisés sous diverses influences DA-70	7,800	6,900

<u>Name</u> <u>Department</u> <u>University</u>	<u>Project Title</u>	<u>Requested</u> \$	<u>Recommended</u> \$
Leeson, T.S. Anatomy, Alberta(Ed.)	Histology and fine structure of ducts of salivary glands of the rat DA-139	3,500	2,800
Listgarten, M.A. Periodontol., Toronto.	1. Distribution of oral spirochaetes in perio- dental disease 2. Ultrastructural characteristics of the epithelial cuff and gingival crevice area DA-136	12,224	9,700
McMurchy, K.A. Oral Pathol., Alberta(Ed.)	Investigations into the pathology of the giant cell reparative granu- loma DA-69	4,600	2,000
Saunders, R.L. de C.H. Anatomy, Dalhousie	Microangiographic studies of the monkey and human periodental vessels DA-105	3,170	3,000
Sedlezky, B. Endodontics, McGill	To study the reactions of dental socket tissues of the adult rat to im- planted simulated teflon root tips	600	Nil
Simard-Savoie, S. Dental Surg., Montreal	Etude expérimentale et clinique des propriétés analgésiques des dérivés phénothiaziniques et de nouveaux analgésiques DA-127	7,300	6,000

Name	Department	University	Project Title	Requested \$	Recommended \$
Sperber, G.H.	Dentistry,	Alberta(Ed.)	Influence of ankylosis on jaw growth in the rat DA-137	1,800	1,400
Spouge, J.D.	Oral Biol., Br. Col.		1.Hypersensitivity reactions in mucous membranes 2.Pathogenesis of the pleomorphic salivary adenoma 3.Clinically appropriate pathological problems DA-93	9,575	4,000
Torneck, C.D.	Research & Endodontics,	Toronto	Reaction of rat connective tissue to polyethylene tube implants DA-138	3,057	1,000
Tuba, J.	Biochem., Alberta(Ed.)		1.The influence of intestinal absorption on body fluids 2.Metabolism of the carbohydrate constituents of submaxillary glands and gingival tissues DA-94	10,500	8,400
Youdelis, W.V.	Metallurgy, Alberta(Ed.)		Metallurgical fundamentals of dental alloys and the development of dental materials: amalgam and gold alloys DA-95	5,400	1,050

Special Summer Undergraduate Assistantships

Ellis, R.G., Dean	Summer Undergraduate	2,000	2,000
Fac. of Dentistry	Assistantships		
Toronto	DA-113		

Leung, S.W., Dean Fac. of Dentistry, Br. Col.	Summer Undergraduate Assistantships DA-117	2,000	2,000
Lussier, J.-P., Dean Fac. of Dental Surg., Montreal	Summer Undergraduate Assistantships DA-116	2,000	2,000
MacLean, H.R., Dean Fac. of Dentistry Alberta (Ed.)	Summer Undergraduate Assistantships DA-111	2,000	2,000
McCutcheon, J., Dean Fac. of Dentistry McGill	Summer Undergraduate Assistantships DA-115	2,000	2,000
McLean, J.D., Dean Fac. of Dentistry Dalhousie	Summer Undergraduate Assistantships DA-112	2,000	2,000
Neilson, J.W., Dean Fac. of Dentistry Manitoba	Summer Undergraduate Assistantships DA-114	2,000	2,000

c) Major Equipment Grants

Due to lack of funds, Dr. J.-P. Lussier and Dr. I. Kleinberg agreed to forego consideration of their applications for Major Equipment Grants, in order that additional money might be made available for Operating Grants. These requests are as follows:

Name	Department	University	Equipment	Requested \$	Recommended \$
Kleinberg, I.	Oral Biol.,	Manitoba	Spinco Model E analytical ultra-centrifuge	34,733	Withdrawn

Name	Department	University	Equipment	Requested \$	Recommended \$
Lague, J.-G. & Lussier, J.-P.	Dental Surg., Montreal		Compteur à scintil- lation Nuclear (Model 6810)	7,000	Withdrawn

d) Travel Grants

The Committee then considered, for recommendation only, one application for a Travel Grant, as follows:

Name	Department	University	Purpose of Travel	Requested \$
Hunt, A.M.	Dentistry, Toronto		To participate in a symposium on tooth support at Oxford University, England; 5 - 8 July, 1965	119

The Committee agreed that Dr. Hunt's application be recommended to Council. Payment will be made from Council funds provided for this purpose.

It was moved by Dr. L. E. Francis and seconded by Dr. I. Kleinberg, that grants be awarded in the amounts specified.

CARRIED

During the discussion of applications for grants requested by attending members of the Committee, each member left the room until his case had been discussed and the recommendation made.

13. Reviewers' Assessments

The Committee agreed that certain of the applicants should be made aware of the Reviewers' comments regarding their respective grant proposals.

They recommended that Dr. A.K. Das be informed that, unless his project showed favorable results, and his succeeding applications were more convincingly organized, no further support from the Associate Committee would be forthcoming.

The Committee noted that Dr. W. V. Youdelis would be moving to the University of Windsor in September 1965, and reduced his grant in accordance with his actual needs while at the University of Alberta. It was also agreed that a full outline of the copious Reviewers' remarks regarding his three projects be forwarded to him.

The Committee noted also that Dr. T. S. Leeson's application requested funds to support Dr. C.L. Windsor, who in 1964-65 held a Dental Research Fellowship, in spite of the fact that he was beyond the age limit specified for Fellows.

Although the Committee recognized the purpose of Dr. Leeson's request, they nonetheless felt that a full report of the research done by Dr. Windsor under Dr. Leeson's direction was necessary.

Consequently, funds were encumbered for this grant, pending receipt of such a report, to be sent, when received, to Dr. L. F. Bélanger for assessment and decision regarding release of funds.

14. Allotment for 1965-66

The Committee's budget for 1965-66 was recommended for allocation as follows:

1 Scholarship	\$ 8,800*
2 Associateships	22,800*
15 Fellowships	63,100 (approx.)

7 Term Grants	\$ 64,500
2 Term Supplements	12,000
14 Summer Assistantships	14,000
26 Annual Grants	<u>124,500</u>
Total	309,700

*Plus applicant's share of cost of University Pension Plan, etc.

15. Estimates for 1966-67

The Chairman reported on the procedure followed in presenting Committee estimates for 1965-66. At the request of Council, in September 1964, he had prepared a written outline of the Committee's estimated requirements for the coming year.

Following this report, discussion took place regarding methods of increasing the Committee's allotment; it was agreed that success in this could be achieved primarily by encouraging dental schools to submit applications.

In addition, it was decided that preparation of the figures for 1966-67, when these estimates were requested by Council, would remain the responsibility of the Chairman.

16. Support for Dental Research in Canada

The Committee then briefly discussed the support of dental research in Canada by agencies other than the National Research Council. The following is a summary of such support:

<u>Source of Support</u>	<u>Amount (approx.)</u>
	\$
National Research Council	297,300
Canadian Dental Association	12,735
Dept. of National Health & Welfare	75,892
Ont. Cancer Treatment & Res. Foundation	6,381
Industry	18,638
U.S. Public Health Service	<u>10,500</u>
Total	\$421,446

17. Special Grant to Deans of Dental Schools

The Chairman then drew attention to a letter from Dr. R. M. Grainger suggesting that uncommitted funds be made available to each dental school to finance pilot studies or to cover small expenses of graduate students.

After some discussion, it was decided that the Chairman should explore the possibility of instituting such a "general research" grant with the President of the National Research Council.

18. Department of National Health and Welfare: Report -

Dr. R.A. Connor

Dr. R. A. Connor then reported on the recently established Subcommittee on Dental Research of the Department of National Health and Welfare, summarizing briefly its financial situation and stressing the need for a close relationship between this Subcommittee and the Associate Committee on Dental Research.

He noted that the two Committees shared some of the same members and often the same problems, such as the shortage of funds and the necessity for encouraging applications to increase the allotment. However, unlike the Associate Committee, the Department of National Health and Welfare supports primarily clinical and public health research, specifically directed towards the improvement of the dental health of the people of Canada. Applications are channelled through the provincial governments, and recommendations made by the Subcommittee are presented to the Research Advisory Committee of the Dominion Council of Public Health.

After describing briefly the type of grants available, Dr. Connor noted that more formal information would soon be forthcoming concerning possibilities offered by the Department of National Health and Welfare.

Following a brief discussion on the support of clinical research, it was agreed that, until such information was available, no definite recommendations would

be publicized concerning submission of applications for the support of clinical research.

19. Forms and Regulations

a) Graduate Dental Research Fellowships

After deliberation, the Committee decided to insert an additional sentence into paragraph 7 of the Regulations governing Dental Research Fellowships, requesting from the candidate's supervisor an outline of the former's proposed research program during tenure of the Fellowship.

b) Grants in Aid of Research

The application form for grants-in-aid was altered under "Amount Requested", to include "first year".

20. Membership of the Committee

The Chairman informed the Committee;

a) that two members, Dr. R. M. Grainger and Dr. J.C. Wilt had completed their first three-year term and were eligible for re-appointment for a second three-year term.

Dr. J. C. Wilt declined re-appointment.

It was moved by Dr. I. Kleinberg, and seconded by Dr. L. E. Francis, that Dr. R. M. Grainger be re-appointed for a second three-year term, expiring 31 March, 1968.

CARRIED

b) that five new members should be appointed to replace:

Dr. L. F. Bélanger
Dr. L. E. Francis

Dr. A. G. Gornall
Dr. I. Kleinberg

who were retiring after six-year terms, as of 31 March 1965;

and Dr. J. C. Wilt.

The following new members were duly nominated and elected:

Dr. G. H. Beaton
School of Hygiene,
University of Toronto

Dr. C. M. Dowse,
Dept. of Oral Biology,
University of Manitoba

Dr. G. E. Connell
Dept. of Biochemistry,
University of Toronto

Dr. D. P. Hill,
Dept. of Pathology,
University of Ottawa

Dr. G. J. Parfitt,
Dept. of Oral Medicine,
University of British Columbia

It was moved by Dr. I. Kleinberg and seconded by Dr. D. Bélanger, that the above new members be appointed for a three-year term expiring 31 March 1968.

CARRIED

c) that his (Dr. J.-P. Lussier's) term as Chairman expired 31 March 1965.

It was therefore moved by Dr. R. M. Grainger and seconded by Dr. I. Kleinberg, that Dr. J.-P. Lussier be reappointed Chairman for the duration of his term as member of the Associate Committee, i.e. a further two years, terminating 31 March 1967.

CARRIED

The Committee also agreed that the appointment of new members to the Executive to replace Drs. Francis, Kleinberg and Gornall be left to the Chairman.

21. Suggestion Concerning the Development of Scientists for Research Careers in Dentistry

"Whereas the pace of research has become so rapid and the academic requirements so great; and whereas a regular course in Dentistry usually fails to provide sufficient training in the basic scientific disciplines for a research career;

It is recommended that at one or more universities in Canada, there should be discussions between the Faculty

of Dentistry and the Faculty of Arts and Sciences to consider the possibility of options in the Honours Science course which would permit students graduating from this course to apply for entrance to the Second Year of the course leading to the D.D.S. degree.

The combined Science and Dentistry program would thus be completed in seven years. The precedent already exists in some schools in the form of a Bachelor of Science and Medicine Course which also can be completed in seven years.

Graduates of such a program would have the requisite background for training in research to the Ph.D. level. They would also have a knowledge of Dentistry and an appreciation of dental research problems."

The Committee considered this recommendation, proposed by Dr. A. G. Gornall, and after deliberation, agreed that it was worthwhile following up wherever possible.

However, since specific action with regard to the individual schools is outside the Committee's Terms of Reference, it was decided that the recommendation should be brought to the attention of the Council on Dental Education of the Canadian Dental Association, in order that it might receive the widest possible circulation.

22. Recommendation No. 177: Report of the Hall Commission

With regard to Recommendation No. 177 of the Report of the Royal Commission on Health Services, the Associate Committee then turned its attention to a consideration of the possible future of the Committee within the framework of the proposed Health Sciences Research Council, and of possible preparations by dental researchers for the establishment of such a body.

Dr. K. J. Paynter suggested that a guide for the organization of a Division within this Council,

responsible for Dental Research, and an outline of principles to govern the awarding of future grants in dentistry, be prepared, to be used by any group commissioned to set up the Health Sciences Research Council.

It was agreed that a Committee composed of representatives of the Canadian Dental Association and the Department of National Health and Welfare, together with the Chairman of the Associate Committee on Dental Research, should work together in the preparation of this document, and that a report of their conclusions be presented to the Associate Committee.

23. Date of the Next Meeting

The Committee agreed that the Seventeenth Meeting of the Executive would be held in the early fall of 1965; and that the Thirty-Second Meeting of all members of the Committee at approximately the same time in the spring of 1966.

The meeting adjourned at 5:00 p.m., 11 March 1965.

24. Publications of Grantees: 1964

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- Johnson, R.H. The Tetracyclines: A Review of the Literature-1948 Through 1963. J. Oral Therapeutics & Pharmacol. 1:2: 190-217. 1964.
- Johnston, M.C. Derivatives of the Cranial Neural Crest in the Chick Embryo. Anat. Rec. 148: 295. 1964.
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- Paynter, K.J. and Hunt, A.M. Morphogenesis of the Rat First Molar. Arch. Oral Biol. 9: 611-626. 1964.
- Pritchard, E.T. and Nichol, N.E. Cholesterol Esterase Activity in Developing Rat Brain. Biochem. Biophys. Acta. 84. 1964.
- Sandham, H.J. and Kleinberg, I. Effect of Glucose Concentration on Carbohydrate Storage by Salivary Sediment. J. Dent. Res. 43: 842. 1964.
- Simard-Savoie, S., Bloomfield, S., Bernier, J. et Tetreault, L. Etude Clinique des Propriétés Analgésiques de la Morphine et du Placébo sur la Douleur Chronique. Union Med. du Canad. 93: 61. 1964.

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4. Dr. R.V. Blackmore,
Faculty of Dentistry,
University of Alberta,
Edmonton, Alberta.

11 March 1966

*5. Dr. L.E. Francis,
Department of Clinical Dentistry,
McGill University,
Montreal, Quebec.

11 March 1966

*6. Dr. R.M. Greninger,
Division of Dental Research,
University of Toronto,
Toronto, Ontario.

11 March 1966

7. Dr. A.M. Hunt,
Faculty of Dentistry,
University of Toronto,
Toronto, Ontario.

11 March 1966

Associate Committee on Dental Research

Membership

1964-65

Term to Expire

- *1. Dr. J.-P. Lussier, Chairman,
Faculty of Dental Surgery,
University of Montreal,
Montreal, Quebec. 31 March 1967
2. Dr. B.G. Ballard, ex officio,
President,
National Research Council,
Ottawa, Ontario.
3. Dr. R.F. Farquharson, ex officio,
Vice-President, Medical Research Council,
Department of Medicine,
University of Toronto,
Toronto, Ontario.

Representing the Dental Profession

4. Dr. R.V. Blackmore,
Faculty of Dentistry,
University of Alberta,
Edmonton, Alberta. 31 March 1966
- *5. Dr. L.E. Francis,
Department of Clinical Dentistry,
McGill University,
Montreal, Quebec. 31 March 1965
- *6. Dr. R.M. Grainger,
Division of Dental Research,
University of Toronto,
Toronto, Ontario. 31 March 1965
7. Dr. A.M. Hunt,
Faculty of Dentistry,
University of Toronto,
Toronto, Ontario. 31 March 1966

Term to Expire

*8. Dr. I. Kleinberg, 31 March 1965
Department of Biochemistry,
University of Manitoba,
Winnipeg, Manitoba.

9. Dr. S. Wah Leung, Dean, 31 March 1966
Faculty of Dentistry,
University of British Columbia,
Vancouver, B.C.

Representing the Royal Canadian Dental Corps,
Department of National Defence,

10. Brig. K.M. Baird, ex officio,
Director General of Dental Service,
Royal Canadian Dental Corps,
Department of National Defence,
Ottawa, Ontario.

Representing the Division of Dental Health,
Department of National Health and Welfare,

11. Dr. R.A. Connor, ex officio,
Chief, Dental Health Division,
Department of National Health and Welfare,
Ottawa, Ontario.

Representing Dental Research,
Department of Veterans' Affairs,

12. Dr. D.M. Tanner, ex officio,
Director of Dental Research,
Department of Veterans' Affairs,
Ottawa, Ontario.

Representing the Basic and Medical Sciences

13. Dr. L.F. Bélanger, 31 March 1965
Department of Histology,
University of Ottawa,
Ottawa, Ontario.

* Member of the Executive

Term to Expire

- *14. Dr. A.G. Gornall,
Dept. of Pathological Chemistry,
University of Toronto,
Toronto, Ontario. 31 March 1965
15. Dr. J.C. Wilt,
Department of Bacteriology,
Medical College,
University of Manitoba,
Winnipeg, Manitoba. 31 March 1965
16. Dr. C.P. Leblond,
Department of Anatomy,
McGill University,
Montreal, Quebec. 31 March 1966
17. Dr. A.J. Rhodes,
School of Hygiene,
University of Toronto,
Toronto, Ontario. 31 March 1966
18. Dr. F.W. Fyfe,
Department of Anatomy,
Dalhousie University,
Halifax, N.S. 31 March 1967
19. Mrs. J.A. Van Laer, Secretary,
National Research Council,
Ottawa, Ontario.

20-24. Deans of Dental Schools (who are not members of the Committee)

20. Alberta	-	Dr. H.B. MacLean
21. Dalhousie	-	Dr. J.D. McLean
22. Manitoba	-	Dr. J.W. Bellenden
23. McGill	-	Dr. J. MacIntosh
24. Toronto	-	Dr. E.G. Ellis

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* Member of the Executive

Distribution

(i) to members of the
Associate Committee on Dental Research

- | | |
|--------------------------------------|------------------------|
| 1. Dr. J-P. Lussier, <u>Chairman</u> | 11. Dr. R.M. Grainger |
| 2. Brigadier K.M. Baird | 12. Dr. A.M. Hunt |
| 3. Dr. B.G. Ballard | 13. Dr. I. Kleinberg |
| 4. Dr. L.F. Bélanger | 14. Dr. C.P. Leblond |
| 5. Dr. R.V. Blackmore | 15. Dr. S. Wah Leung |
| 6. Dr. R.A. Connor | 16. Dr. A.J. Rhodes |
| 7. Dr. R.F. Farquharson | 17. Dr. D.M. Tanner |
| 8. Dr. L.E. Francis | 18. Dr. J.C. Wilt |
| 9. Dr. F.W. Fyfe | 19. Mrs. J.A. Van Laer |
| 10. Dr. A.G. Gornall | |

(ii) to other persons

20-24. Deans of Dental Schools (who are not members of the Committee)

- | | | |
|---------------|---|-------------------|
| 20. Alberta | - | Dr. H.R. MacLean |
| 21. Dalhousie | - | Dr. J.D. McLean |
| 22. Manitoba | - | Dr. J.W. Neilson |
| 23. McGill | - | Dr. J. McCutcheon |
| 24. Toronto | - | Dr. R.G. Ellis |

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APPENDICES

ASSOCIATE COMMITTEE ON DENTAL RESEARCH

Progress Reports
1964-65

<u>Grantee</u>	<u>Project</u>	<u>Appendix</u>
Blackmore, R.V.	DA-102	A
Burgess, R.C.	DA-130	B
Cleall, J.F.	DA-131	C
Copp, D.H.	DT-59	D
Dawes, C.	DA-122	E
Dowse, C.M.	DA-81	F
Edmund, A.G.	DA-104	G
Grainger, R.M.	DA-123	H
Hamilton, I.R.	DA-124	I
Johnston, M.C.	DA-125	J
Kasloff, Z.	DA-90	K
Khairat, O.	DA-101	L
Lussier, J-P. & Lague, J-G.	DA-70	M
Mezl, Z.	DA-79	N
Paynter, K.J. & Hunt, A.M.	DT-72	O
Pritchard, E.T.	DA-109	P
Saunders, R.L.de C.H.	DA-105	Q
Simard-Savoie, S.	DA-127	R

mod

CHARGE

Project

D. J. O'Brien

Project

Appendix

DA-93

S

DA-128

T

DA-94

U

DA 95

DA-95

V

APPENDIX A

ANNUAL REPORT

RESEARCH GRANT:

N.R.C. -DA-102

INVESTIGATOR:

R.V. Blackmore
University of Alberta

Title:

An investigation of the factors influencing the propagation of Herpesvirus hominis in animal cell cultures and in experimental animal infections.

Period:

Jan. 1964 to December 1964.

Summary:

- A. Monolayer cultures of mouse fibroblasts (Strain L929) were incubated with heat-inactivated (37°C, 72 hr.) Herpesvirus hominis. Such treatment rendered the cells resistant to a subsequent challenge with infectious herpesvirus. The resistance was manifest in two ways. First, the usual cytopathic effect was minimized, and second, the herpesvirus yield was markedly reduced.

Ribosenucleic acid has been prepared from heat-inactivated herpesvirus with a view to determining whether or not its activity is similar to that of heat-denatured whole virus in causing viral interference.

- B. It has been determined that Herpesvirus hominis persists in the tissues of 2 day old rabbits which have experienced a transplacental infection with this virus. Viral antigen can be demonstrated in tissue sections by the indirect fluorescent antibody technique. However, infectious virus could not be recovered from homogenates of samples of the same organs in which specific fluorescence was found. Nevertheless, when explant cultures of kidney tissue from these experimental animals were maintained in culture for six days, Herpesvirus hominis was identified in the culture fluids.

APPENDIX B
ANNUAL REPORT

RESEARCH GRANT: DA-130

INVESTIGATOR: R.C. Burgess
University of Toronto

TITLE: Further studies on proteins in developing enamel

Although this is a 'New Project' in terms of N.R.C. Grants, we have already done some research in this field. Related findings to date are:-

1. Identification and quantitation of carbohydrate components in mature enamel. (17)
2. Confirmation of organic matter loss from developing enamel and demonstration that this is due primarily to loss of 'soluble' protein. (7)
3. Demonstration that developing bovine enamel is composed of two major and at least three minor protein entities. (10) Evidence that all of these proteins decrease in concentration as mineralization proceeds (7), though probably at differing rates.
4. Isolation in homogeneous state of the two major enamel proteins, and proof of their individuality on the basis of amino acid composition and molecular weights (7,23).
5. Preliminary studies on the concentration dependence of molecular weight for one of the major enamel proteins. Indications that this protein can spontaneously aggregate when in concentrated aqueous solution. (23)

2. (b) New Project

2. (c) Pertinent references - these have all been cited or discussed in previous sections. See Section 1(a).

APPENDIX C
ANNUAL REPORT

RESEARCH GRANT: DA-131
INVESTIGATOR J. F. Cleall

A. Summary

1. Research grant title and date:
"Growth, and Growth Deviations of the Cranio-facial skeleton".
December 4, 1964.
2. Investiagtors and sponsoring institution:
J.F. Cleall, University of Manitoba, Faculty of Dentistry.
3. Period covered by this report:
24th June, 1964 to December 4th, 1964. (This grant was awarded following a late application for research support, the investigator having only recently arrived in Canada).
4. Summary statement:
A multiple bone marking technique developed by the principal investigator to study the longitudinal growth of bones in experimental animals has been used to consider growth and growth deviations in the cranio-facial skeletal structures of the rat. A series of young growing animals were serially injected with bone marking agents and were concurrently radiographed (using a specially constructed rat radiographic cephalometer). Serial ground sections of the cranio-facial skeletal structures of each animal are being cut and the information from the specific growth sites is being compared with the cephalometric data.

The cephalometric data has been analyzed but at this stage only a subjective appraisal has been made of the sections containing the bone marking agents. This material will be analyzed in an objective manner by measurement at a later date and the results will be correlated with the cephalometric data. The subjective appraisal however, has shown quite conclusively that many of the growth changes considered to be taking place as a result of the cephalometric appraisal are complete misinterpretations. The assessment of the bone marking agents has allowed many interpretation errors inherent in any cephalometric technique to become apparent.

A second study has recently been undertaken to examine the effect on the growth of the cranio-facial skeleton following surgical interference with the muscles of mastication. To this end, a series of rats have received bone marking agents to follow the skeletal growth changes after surgical resection of the temporal muscle attachment at the nuchal crest.

APPENDIX D

PROGRESS REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

National Research Council of Canada

Grant DT-59

Title: PHOSPHORUS DEFICIENCY--Effects on bones and teeth.

Grantee: D. Harold Copp

Department of Physiology

University of British Columbia

Date: December 31, 1964.

SUMMARY

The work in this laboratory supported by term grant DT-59 from the Associate Committee on Dental Research has been part of a general study of factors affecting calcium homeostasis, with particular emphasis on the effects of dietary alterations in calcium and phosphorus. During the current year, one paper acknowledging this support is in press (Copp, D.H. "The hormones of the parathyroid and calcium homeostasis" in The Parathyroid Glands: Ultrastructure, secretion and function, University of Chicago Press).

The current research is concerned with the effect on parathyroid function of restricting young (90-100 g) rats to a low phosphate diet (0.375% Ca, 0.021% P) for a short period (3 days). An ultra-micro method for calcium analysis using glyoxal bis(2-hydroxyanil) originally developed by C.H. Dowse has made possible serial determinations of calcium in the same rat, since only 0.02 ml of serum is required for each determination.

After 3 days on the low phosphate diet, the plasma calcium increased from 9.582 ± 0.073 mg-% to 11.664 ± 0.051 mg% (a highly significant increase) while the inorganic phosphate level dropped from 8.543 ± 0.102 mg-% to 5.034 ± 0.286 mg-% (also highly significant).

The rats were then parathyroidectomized by electrocautery or sham operated by exposing and cauterizing lightly the lower pole of the thyroid. Eleven of the parathyroidectomized rats were injected ip with 9.7 mg P per 100 g as isotonic potassium phosphate. A similar number were injected ip with isotonic saline. The sham operated rats were treated similarly. Blood samples were collected from the tail at 0, $\frac{1}{2}$, 1, 3, 6, 9 and 24 hours after the injection and were analyzed for Ca and P. A small transient fall in plasma calcium occurred in both groups injected with saline, but there was no significant difference between the parathyroidectomized and the control groups. The plasma calcium had returned to 11.614 ± 0.127 at 24 hours, and it is felt that the transient fall may have been due to anesthetic and failure to eat during the day of operation.

In the rats of both groups injected with phosphate, there was an immediate fall in serum calcium of 4-5 mg-% within an hour, and recovery was very slow. There was no significant difference between the parathyroidectomized and sham operated groups.

In contrast, in the case of rats fed a control diet (0.383% Ca, 0.400% P) for three days, there was no significant change in the calcium and phosphate levels in serum. In the sham operated control rats fed this diet, administration of saline ip had no effect on serum calcium or phosphate while administration of isotonic phosphate ip resulted in a transient fall in serum calcium within an hour, with recovery of the normal level in 3 hours. Administration of P to the parathyroidectomized animals resulted in a prompt and sustained reduction in serum calcium.

It is evident that in the rats fed the control diet, the parathyroids are exercising efficient homeostatic control of hypocalcemia, while this function is lost in the animals fed the low phosphate diet for 3 days. This suppression of normal parathyroid function may be due to the hypercalcemia produced by the low phosphate diet.

APPENDIX E
ANNUAL REPORT

RESEARCH GRANT: DA-122

INVESTIGATOR: C. Dawes
University of Manitoba

TITLE: Factors influencing the secretion and precipitation of salivary proteins

I have worked on project no. DA-122 since September 1964 when I took up my appointment at the University of Manitoba. Although none of my studies are complete, some data has been collected. However, conclusions drawn from the data are essentially tentative in nature.

1. The effects of prolonged stimulation upon human parotid salivary flow rate and protein concentration.

By continuous gustatory stimulation with "Sour Lemon Drops", a high rate of flow of about 1 ml/min/gland could readily be maintained for about one hour. Three experiments were carried out on the same subject. In the first experiment, essentially no change with time could be detected in the protein concentration in twelve successive 5 minute samples of saliva. In the second and third experiments, a slight decrease in protein concentration occurred in the final few samples. Paper electrophoretic analysis (Dawes, C. 1963. Arch. oral Biol. 8, 653-656) of the successive 5 minute samples indicated that no change had occurred in the relative proportions of the different protein components throughout the one hour period. The results are in marked contrast to studies on dog saliva by Anrep (J. Physiol. 54, 319-331 (1921)) and Burgen et al (Physiology of the Salivary Glands, 1961, p. 182 by Burgen and Emmelin, Arnold, London.). These workers found a very marked decrease in total salivary protein concentration upon prolonged stimulation of parotid secretion.

2. A comparison of the composition of human parotid saliva secreted in response to either "Sour Lemon Drop" stimulation or to pilocarpine HCl.

Pilocarpine is a parasympathomimetic drug which acts directly on the salivary glands to cause secretion. Ingestion of pilocarpine HCl caused an increase in parotid salivary flow rate to levels as high as 1.1 ml/min/gland. As with gustatory stimuli there was little evidence of a decreased salivary protein concentration with prolonged stimulation of the gland. The proportions of the different types of salivary protein, as analyzed by paper electrophoresis, did not change throughout the period of stimulation and were essentially similar to the proportions of proteins secreted in response to gustatory stimuli.

Studies on three different subjects showed that at any given flow rate, parotid saliva secreted in response to pilocarpine contained a higher concentration of protein than did saliva secreted in response to a gustatory stimulus. This observation may partially explain the findings that pilocarpine-stimulated rat and monkey saliva (Arch. oral Biol. 7, 621-631; I.A.D.R. preprinted abstracts No. 345, 1963) contains considerably higher levels of protein than values reported for human saliva (J. dent. Res. 27, 603-608, 1948).

Studies on some of the inorganic constituents of saliva showed that at any given flow rate, pilocarpine-stimulated parotid saliva contained a higher concentration of calcium, a lower concentration of sodium and potassium but a similar concentration of phosphate to saliva secreted in response to a gustatory stimulus.

Further studies are needed to confirm these observations and also to determine whether the higher calcium concentrations in pilocarpine-stimulated saliva can be accounted for by increased binding by the higher protein concentrations.

APPENDIX F

PROGRESS REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

1964

GRANT TITLE: DA-81

"Metabolism of Periosteum"

GRANTEE: C.M. Dowse

University of Manitoba

Certain aspects of the in vitro metabolism of carbohydrate by calvaria have been studied. There is present in the tissue an endogenous substrate, which could not be depleted by preincubation. It has been tentatively identified as glycogen. Glycogen breakdown appeared to take place only during the first 30 minutes of incubation. This finding suggests that complications due to its presence may be avoided by addition of glucose-C¹⁴ after an initial period of incubation with unlabelled substrate.

Assessment was made of the effects of (i) variation in the concentrations of glucose and inorganic phosphate, (ii) depletion of the endogenous substrate, and (iii) anoxia, upon (a) the amount of glucose-U-C¹⁴ utilized and the amount appearing in lactate, and (b) the contribution of glucose-C¹⁴ and unlabelled endogenous sources to the total lactate production. The results were compatible with the hypothesis that control upon glycolysis in the tissue is principally exerted through the activity of phosphofructokinase.

Changes in glucose utilization and lactate production produced

C. M. Dowse

by a partially purified preparation of parathyroid hormone suggested that the effects of the hormone upon glycolysis were secondary to a more primary action of the hormone upon some other aspect of carbohydrate metabolism.

APPENDIX G
ANNUAL REPORT

RESEARCH GRANT: DA-104

INVESTIGATOR: A. Gordon Edmund
Royal Ontario Museum
University of Toronto

TITLE Dynamics of Tooth Replacement in
Modern Reptiles

The programme of observing the rapidly replacing dentitions of living reptiles was continued, resulting in over 1200 radiographs. More detailed radiographs are now being produced by the recently installed General Electric R2 machine. Approximately thirty reptiles have been maintained at any one time, with over twenty yielding long term series of observations. Almost all show that replacement of teeth is continuous, with the life of each tooth being about 5 or 6 months. Approximately twenty-five teeth per jaw quadrant are replaced each year, and the progress of waves of replacement is illustrated for one specimen. These data were used to construct a graphic model of an iguana jaw which agrees with the Zahnreihe theory outlined by Edmund (1960, 1962) and with radiographic and histological observations.

Short series of functional teeth, parts of the dental lamina, or both, were surgically extirpated from a number of dentitions. Preliminary results indicate a rapid repair of the damage with restoration of the regular replacement rhythm.

Because of the essentially long-term nature of the study, analysis of the results is premature. However, the rate and remarkably regular sequence of tooth replacement has been established for a wide spectrum of animals and analysis of the data currently being collected should be complete by the end of 1965.

APPENDIX H

ANNUAL REPORT - DA-123

1. Statistical Consultation and Analysis Unit.
2. R.M. Grainger, D.D.S., D.D.PH., M.Sc.D.
Faculty of Dentistry,
University of Toronto.
3. Period March 1st 1964 to December 21st 1964.

Summary Statement:

This application is for support of I.B.M. equipment rental in the Research Division of the Faculty of Dentistry, University of Toronto. The applicant's work as professor of Epidemiology and Statistics and as statistical consultant to a wide range of research projects entails much data processing. The calculations are done on the I.B.M. 7094 at the Institute of Computing Science on the University Campus, but the work of preparing the card decks and developing programs must be done in the Dental Building which is some blocks off campus.

Research made possible in the last year by this grant include: (1) The Dental Student Aptitude Testing Studies carried out for the Council of Dental Education. (2) A study of the Potential Technical Aptitude of Dental Hygiene Students carried out for the Dental Faculty Council. (3) An analysis of a five year study of the Dental Caries Swab Test. (4) continuing work on the Epidemiology of tooth morphology. (5) analysis of animal nutrition experiment data in connection with the application from Dr. K.J. Paynter supported by N.R.C. (6) processing data from the amino-acid analysis equipment purchased under the Nikiforuk application. (7) research of developing a set of computer programs suitable for analysis of dental research data. (8) development of a program for processing mark sense cards used in objective examinations. (9) analysis of growth and development data in connection with the application from D. Woodside supported by N.R.C. (10) analysis of marks from the National Dental Examining Board. (11) analysis of data for half a dozen graduate students in orthodontics, pedodontics, periodontics and public health.

APPENDIX I

PROGRESS REPORT (DA-124)

Title: Anaerobic carbohydrate metabolism by Streptococcus salivarius.

Date: December 23, 1964.

Investigator: Dr. Ian R. Hamilton,
University of Manitoba, Winnipeg.

Period Covered: October 1, 1964 to December 23, 1964.

This report represents the beginning of a long term project to isolate and characterize those microbial components of the oral cavity that are responsible for the pH and related changes in dental plaque and salivary sediment. Once isolated in pure culture, these organisms will be examined for their basic biochemical capabilities and then combined to form a "model" ecological population having the characteristics of the "natural" flora of the oral cavity. In this manner, fundamental processes related to such oral diseases as dental caries and periodontal disease can be investigated.

Streptococcus salivarius, likely an important component for the above model, was isolated from dental plaque on the anterior teeth, the surface of the tongue and from non-stimulated saliva. Two distinct strains were found and the one that more closely resembled the published description of Streptococcus salivarius was selected for biochemical study.

Studies on the anaerobic fermentation of glucose by this organism

I.R. Hamilton

confirmed its homofermentative nature, i.e., lactic acid was the only product of glucose degradation. At the same time as this acid was formed from glucose, other glucose molecules were used for the biosynthesis of cellular constituents. Cells of Streptococcus salivarius, grown in a 0.1% glucose medium, incorporated into cellular material 13-14% of the metabolized glucose when incubated in buffer in the absence of nutrients. The addition of nutrients to these cells tripled the rate of glucose metabolism, increased the absolute amount of glucose carbon assimilated, but reduced the amount incorporated as a fraction of the total glucose utilized. Cells grown on 1.0% glucose, on the other hand, had greater metabolic rates, incorporated more glucose carbon into cell material, but assimilated less as a fraction of the total glucose metabolized than the 0.1% glucose-grown cells. The addition of nutrients to the 1.0% cells also stimulated metabolism, but reduced the glucose-carbon uptake.

Experiments are in progress to determine whether the intracellular material formed is polysaccharide and, if so, whether this stored material can be subsequently utilized by the cell in the absence of exogenous glucose.

APPENDIX J

ANNUAL REPORT - DA-125

1. Grant Title: Studies on the Early Embryological Development of the Face. Date: 1964 - 5.

2. Investigator: M. C. Johnston.

Supporting Institution: University of Toronto Faculty of Dentistry

3. Period Covered by Report: April /64 - Dec. /64.

4. Summary Statement:

A reasonably well-equipped laboratory has been established and is now operating satisfactorily. The personnel include a full-time M.Sc.D. student and a full-time technician.

The research is progressing satisfactorily. The project being conducted by the graduate student, Dr. R.D. Haselton, is nearing completion. It is concerned with the derivatives of the occipital somites whose cells were labelled with tritiated thymidine, and the migrations and fates of the cells determined radioautographically. Besides contributing to the expected tissues in their immediate vicinity these cells participated in the formation of the meninges and to the hypoglossal cord which migrates into the tongue where it gives rise to the hypoglossal musculature.

In contrast to Dr. Haselton's study the remaining projects are more closely inter-dependant. A technique of direct chromosome analysis of chick embryo tissues has been worked out and applied to facial tissues in embryos which had received neural crest grafts from embryos of the opposite sex. The results are in a preliminary stage of analysis.

Various groups of cells in the facial region of the developing chick embryo, whose origins had been determined from earlier studies, were characterized histochemically. This additional method of analysis is now being applied to the study of normal and abnormal facial development in mammalian embryos.

APPENDIX K

ANNUAL REPORT

RESEARCH GRANT: DA-90

INVESTIGATOR: Z. Kasloff
University of Manitoba

TITLE: Metallographic studies of soldered joints
and cast gold parent metals

May 15, 1964----- December , 1964.

This study, the first phase of the project, concerned itself with the effect on the microstructure of cast gold parent metals when subjected to 3 different fusing range solders. The property of hardness on the parent metals and influences on it by the application of the different heat ranges was also included in this portion of the investigation. Sixty cast gold rods, to serve as test specimens, were uniformly made under controlled conditions, using type C casting gold ingots obtained from one manufacturer. Initial experiments were conducted to determine the effect on the microstructure of the cast metals when 2 time intervals of bench-cooling preceded the water quenching after casting. Standard metallurgical technical procedures were employed in specimen preparation for metallographic examination and photography. Controlled conditions for soldering were used, the only variables being the solders for joining the parent metals. Hardness determinations were made on all specimens at specified locations for comparative evaluation. Results indicated that longer bench-cooling periods prior to water quenching decreased hardness in the cast structures and induced larger grain formation. The two higher fusing-range solders induced grain formation in the solder areas. Increased hardness was evident when lower soldering temperatures were employed.

APPENDIX L

SUMMARY OF

December 26, 1964

The Final Progress Report (Grant DA-101)

Name : Omar KHAIRAT,
:
Institution : Faculties of Medicine & Dentistry, University of Manitoba
Title of Project : Post-extraction bacteraemia, with special emphasis on the anaerobes, and an antibiotic active against all the aerobes and all the anaerobes isolated from the circulating blood.

- - - - -

One hundred post-extraction blood cultures (and another 100 pre-extraction blood cultures) were taken from the same 100 patients without an antibiotic cover, to establish a base line surveying the types of aerobes and anaerobes encountered in such bacteraemias.

The positive post-extraction blood cultures in this survey were 64%, and more than one type of organism was isolated from one patient in the majority of cases. The strains isolated were 81 anaerobes and 64 aerobes. The anaerobes included anaerobic streptococci, Fusiformis, Bacteroides melaninogenicus, other Bacteroides species, Veillonella orbiculus and anaerobic corynebacteria. The aerobes were: viridans streptococci, Str.pyogenes, corynebacteria, Neisseria, Staph.aureus, Nocardia and Klebsiella aerogenes. None of the pre-extraction cultures yielded any growth.

Of the strains isolated, 58 aerobes and anaerobes were in-vitro tested for sensitivity against 22 antibiotics in the lowest concentration used. Four antibiotics were found to inhibit the growth of almost all the aerobes and all the anaerobes whether Gram positive or Gram negative.

One antibiotic of these four was tried on 42 patients, increasing the dose from 250 mg to 1 gramme given 1½ to 4 hours before the extraction, but 16 positive blood cultures were obtained (38%), a reduction from 64% to 38% which was statistically insignificant.

A second antibiotic was tried on 100 patients and this time only two positive blood cultures were obtained, both viridans streptococci. In the present work where a large number of patients and an equally large number of controls were tested with an optimum technique both as regards the blood culture medium and perfect anaerobiosis, and where the highest number of anaerobes ever isolated in a series of 64 post-extraction positive blood cultures, with 58 strains tested against 22 antibiotics, the splendid result obtained makes endocarditis complicating exodontia, a matter of the past. The work is under publication and reprints will be sent to the National Research Council of Canada.

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During 1964 work was done concurrently on (1) Studies on an optimum general-purpose culture medium for aerobes and anaerobes. (2) Studies on an optimum blood culture medium. (3) Studies on a new species of Fusiformis never before isolated from the circulating blood following tooth extraction.

It is hoped papers on these subjects will be published during 1965.

APPENDIX M

COMPTE-RENDU ANNUEL DA-70

1. Titre: Antagonisme entre le fluor et le citrate au niveau des tissus en période de calcification sous des conditions où la citricémie est élevée.

Date: Mars 1964.
2. Jean-Paul Lussier et Jean-Guy Laguë,
Université de Montréal.
3. Mars 1964 à décembre 1964.
4. D'une part nous savons qu'il existe une relation inverse entre le contenu en fluor et en citrate dans l'os. D'autre part la ^{qualité} quantité de l'os est affectée par les concentrations respectives de ces deux éléments: une augmentation du citrate diminue la résistance des tissus minéralisés alors qu'une augmentation du fluor augmente cette résistance. Le citrate dans les tissus en période de calcification est doublé lorsque l'animal est placé dans des conditions d'hypercitricémie obtenu après hépatectomie-néphrectomie.

Nous nous sommes proposés de voir si l'accumulation du fluor dans les tissus minéralisés peut empêcher l'augmentation du citrate qui se produit dans ces tissus lorsqu'ils sont exposés à une citricémie élevée.

J.-P. Lussier et J.-G. Lagué

Nous avons utilisé des rats mâles en période de croissance et les avons soumis à un traitement au fluor à raison de 0, 50 et 100 p.p.m. dans leur eau de consommation pendant 25 jours.

Après ce traitement, un certain nombre de rats dans chaque groupe ont été hépatectomisés-néphrectomisés. Le contenu en citrate et en fluor ont été analysé dans le sang, le tibia, la dentine des molaires et des incisives inférieures. Les incisives ont été sectionnées en trois portions: supérieure, moyenne et apicale. Les deux premières portions sont déjà calcifiées alors que la dernière est en formation constante.

Nous avons réalisés les observations suivantes. Au niveau du tibia, le fluor déjà incorporé n'empêche pas l'augmentation de son contenu en citrate dans des conditions d'hypercitrémie. Inversement, dans ces conditions, le citrate ne parvient pas à déloger le fluor déjà incorporé.

Au niveau des molaires et des incisives, il se produit une baisse du citrate d'environ 30 à 50% chez les animaux témoins lorsque le fluor a été préalablement administré à raison de 100 p.p.m. Chez les animaux hépatectomisés-néphrectomisés, cette baisse ne se manifeste que dans la portion apicale des incisives. Donc pendant la courte période de 25 jours pendant laquelle le fluor a été administré, seuls les tissus dentaires ont été affectés dans leur contenu en citrate par ce traitement.

APPENDIX N
ANNUAL REPORT

RESEARCH GRANT: DA-79

INVESTIGATOR: Z. Mezl, University of Montreal

TITLE: Experimental modifications of the permeability of the superficial zone of enamel caries

E X T R A C T

Discussion

This is the second part of a three year project.

The report of this second part can be divided in three parts:

1. Development of new methods.
 2. Experiments attempting to make permeable carious lesions impermeable.
 3. Experiments attempting to make impermeable carious lesions permeable.
1. The measurements of the rate of the penetration of eosin in carious lesions (chapter 1) were made only as a step to develop the method to be used later. The eosin was later replaced by toluidine blue because this is better visible. The measurement was replaced by photographic recording described in chapter 6 because this allows to get permanent records. Using the slides, the depth of the penetration can be measured later. Details such as particular pathways of penetration which are visible only briefly during the experiment and later covered by deep staining can be studied later on the screen. Details appearing all over the lesion during the entire experiment can be plotted in a single drawing.

The variety in the rate of penetration which is noted in chapter 1 deserves further study but this does not belong under the title of this project. The photographic technique would allow another project, time-lapse cinematography of the penetration of solu-

2. The experiments attempting to make permeable carious lesions impermeable were first continued as in 1963 (chapter 2) and later modified, using a solution of NaF. (chapter 3 and 4). Table 6 gives a summary of the results during 1963 and 1964. Because carious lesions are a very variable material, the results are not suitable for a mathematical evaluation.

The solutions of dental tissues in lactic acid gave the best results. About one lesion out of ten became impermeable, in about half of the lesions the penetration was slower after the treatment with this solution.

3. The experiments attempting to make impermeable carious lesions permeable were continued with the same lactate buffer solution at a pH 5.12 as in 1963 (chapter 5), but partly with the new photographic technique (chapter 6). This technique shows an interesting variety in the rates, patterns and pathways of penetration which deserves further study.

In rare cases, the lesion remained impermeable after 3 hours.

In a few cases, the lesion became easily permeable; the solution reached the full depth of the lesion in about 30 minutes.

In most cases, the solution penetrated into the carious lesion slowly, requiring more than 90 minutes to reach the full depth of the lesion; in many cases, it did not stain the lesion completely during three hours.

The periods required for complete penetration of certain carious lesions are surprisingly long when one considers the periods of acidity which have been measured in the bacterial plaque in situ.

Z. Mezl

Summary

A method has been developed for observation and measuring of the rate of penetration of staining solutions into initial carious lesions of the enamel:

A ground surface is prepared longitudinally through the tooth and through the center of the lesion. This surface is accurately covered with a transparent varnish, resistant and impermeable to the solutions used. Through the varnish, the degree of penetration of the stain entering through the natural surface of the lesion can be observed, measured and photographed with a microscope using reflected light.

Rates of penetration of aqueous solution of eosin from 0 to 31 microns per minute were measured and several types of penetration were distinguished.

In deeper conical lesions, the rate was at first between 10 and 20 microns per minute and later it slowed down to a few microns per minute. In certain cases, deeper parts of the lesion were not stained; in other cases, the stain penetrated to the full depth of the lesion at the same rate.

In superficial lesions, the rate of penetration was usually under 10 microns per minute.

In some cases, the rate of penetration was very irregular, variable or very low, about 1 micron per minute.

Carious lesions which were originally permeable, were treated by 7 different experimental procedures using the following solutions: 1. rehardening solution (Koulourides, Cueto and Pigman), 2. saturated solutions of Ca HPO_4 in lactic acid, 3. saturated

Z Mezl

solutions of dental tissues in lactic acid, 4. saturated solutions of Ca HPO_4 in lactic acid combined with Na F. The permeability of the lesion before and after the experiment were compared.

In about half of the cases, the penetration of staining solutions into the lesion was slower after the experiment than before the experiment.

In about one lesion out of ten the penetration of stain was faster after the experiment.

Certain lesions easily permeable at the beginning have been rendered impermeable. From the lesions treated by the solutions of dental tissues about one out of ten became impermeable.

Carious lesions impermeable to aqueous solution of eosin were treated with lactate buffer solutions at a pH of 5.12. This solution penetrated into most lesions during periods varying between 90 minutes and 3 hours. In many cases the solution did not penetrate to the full depth of the lesion. Few lesions remained impermeable, few became easily permeable.

APPENDIX O

REPORT TO THE ASSOCIATE COMMITTEE ON DENTAL RESEARCH

NATIONAL RESEARCH COUNCIL

January 1, 1965

Subject: Factors influencing tooth morphology and caries susceptibility

Grantees: K.J. Paynter and A.M. Hunt

Project: DT72

Amount: Three-year Term Grant March 1962 to
March 1965.
\$8,000 per year

SUMMARY

This work has progressed along two main lines. We have continued to study the effect of nutritional elements such as fluoride, vitamin D, and calcium and phosphorous on the development of tooth form and on caries susceptibility.

New information has been obtained on the interaction of fluoride with vitamin D. Some evidence has been obtained that both an insufficient amount and an excess of vitamin D during the tooth formative period may be detrimental to dental health. It also appears that excessive levels of calcium and phosphorous in diets may affect teeth adversely.

The second main line of study has attempted to elucidate the mechanisms whereby these dietary effects are exerted on teeth. This has led to a more basic study of ontogenesis of molar teeth in rats utilizing both reconstructions of developing teeth, and autoradiographs following H₃-labelled thymidine administration to follow mitosis and cell migration in the enamel organ. A better understanding has resulted of the relation

between cells and tissues in the enamel organ as tooth form becomes established. Additional studies are in progress to follow up ideas originating in this basic study.

K. J. Paynter & A.M. Hunt
Department of Biology
Faculty of Science
University of Cambridge

Summary of the Report

Examination of 1944 types present in submaxillary salivary gland (SMG) of the rat have been made. Further studies on the pattern and extent of labelling of lipids by radioactive acetate and glycerol have been made. SMG lipids were well labelled from both precursors. The results with glycerol-1-3-14 demonstrated that a wide spectrum of lipid types could be synthesised by the gland. Several classes of sphingolipids appear to exist in the SMG but one class is deficient.

Examination of SMG lipids with radioactive 14C and 3H precursors showed similar labelling from both precursors. This would suggest that the SMG is capable of synthesising a wide range of lipid types and that the precursors are well incorporated.

Brain sphingolipids can be formed from either phosphatidylcholine (PC) or sphingomyelin (SM) but the former appears to be the main precursor.

Cholesterol esters labelling has been observed to occur in rat brain preparations.

APPENDIX P

PROGRESS REPORT FOR 1964

(Grant DA - 109)

SULFOLIPIDS IN RAT TISSUES

Investigation of Lipid Metabolism in Secreting
Submaxillary Salivary Gland and Brain of the Rat

By

E. Thackeray Pritchard, Ph.D.

Associate Professor

Oral Biology

Faculty of Dentistry

University of Manitoba

Summary of the Report

Examination of lipid types present in submaxillary salivary gland (SMSG) of the rat have been made. Further studies on the pattern and extent of labelling of lipids by radioactive sulfate and glycerol have been done. SMSG lipids were well labelled from both precursors. The results with glycerol-1-C-14 demonstrated that a wide spectrum of lipid types could be synthesized by the gland. Several classes of sulfatides appear to exist in the SMSG but one class is dominant.

Stimulation of SMSG slices with acetylcholine did not influence either sulfolipid labelling from radiosulfate nor total lipid labelling by glycerol-1-C-14. This would suggest that lipid components formed from these two precursors were not directly involved in secretion.

Brain sulfatides can be formed from either phosphoadenosine phosphosulfate (PAPS, active sulfate) or inorganic sulfate but the former compound appears to be a much better precursor.

Cholesterol esterase activity has been observed to occur in rat brain preparations.

APPENDIX Q

Report To The Associate Committee on Dental Research National Research Council of Canada .

DA-105

1. Research Grant Title and Date .

Microangiographic Studies of the Monkey and Human
Peridental Vessels .

Application made Nov.14th,1963. Granted March 5th,1964.

2. Names of Investigators ,and Sponsoring Institution.

R.L.de C.H.Saunders. Anatomy Department ,Dalhousie University

3. Period Covered by Report.

June to December 1964.

4. Summary Statement.

The literature , when reviewed by the grantee for a book (in press), showed that little research had been performed on the blood vessels within the human jaw. Textbooks gave inadequate accounts ,overlooked age changes,and omitted particulars of material examined and sources consulted.

The collection of fresh dentulous human material presents a problem ; it requires time and persistence . So far four monkey,seven human foetal and four adult jaws have been prepared for X-ray microscopy. Intravascular injection with radiopaques followed by contact microradiography provides good overall delineation of the peridental vasculature .The distribution of the alveolar arteries in dentulous and edentulous areas has been recorded ,also the intraosseous vascular mesh pattern. Differentiation of the arteries and veins within the jaw is being attempted with Chromopaques;

new fine particle contrast media of vivid colour whose dilution provides a radiopacity differential . Better anatomical analysis of these small vessels is expected from projection X-ray microscopy ,which gives primary magnification and sharper vessel definition. Hence collected material will first be examined with a low voltage (Mark 11) X-ray microscope ,and later with the new high voltage X-ray microscope (Mark 111, Model XMPJ.; recently constructed under MRC grants).

APPENDIX R
COMPTE RENDU ANNUEL

<u>SUBVENTION:</u>	DA-127
<u>BÉNÉFICIAIRE</u>	S. Simard-Savoie Université de Montréal
<u>TITRE</u>	Étude expérimentale des propriétés analgésiques de la triméprazine, de l'halopéridol et de la triméprimine

La triméprazine est connue depuis longtemps comme antiprurigineux et antitussif. Par une série d'expériences, à l'aide de 4 techniques d'analgésimétrie, nous avons démontré que cette substance possède une activité analgésique spécifique.

L'application de la technique de d'Amour et Smith a donné des résultats négatifs chez le rat. Cependant, à l'aide des techniques de la plaque chauffante et de Bianchi, nous avons déterminé des propriétés analgésiques chez la souris. Les courbes dose-effet sont représentatives à partir de 4 mg/kg. La triméprazine a été comparée à la morphine et à un placebo (test de Bianchi) et nos résultats montrent que l'activité analgésique de la triméprazine (15 mg/kg i.p.) est comparable à celle de la morphine (5 mg/kg i.p.) ($p < 0.05$).

Par la méthode de la stimulation de la pulpe dentaire, chez 11 lapins, le pouvoir analgésique de la triméprazine (15 mg/kg) a été comparé à celui de la morphine (5 mg/kg) et à un placebo (NaCl 0.9%). Les 3 substances ont été administrées par voie intraveineuse. Une technique à double insu a été utilisée. L'analyse de variance, réalisée pour les temps +15 et +60 min. après l'injection, montre que, à la 15e min., la triméprazine est significativement plus efficace que le placebo ($p < 0.001$) mais ne se distingue pas de la morphine de façon significative. Cependant, à la 60e min., la triméprazine est significativement plus efficace à élever les seuils de la douleur que le placebo ($p < 0.001$) et la morphine ($p < 0.01$).

Deux autres substances, l'halopéridol et la triméprimine, ont été étudiées quant à leur activité analgésique possible, au moyen

L'action sédatrice de la triméprazine et de l'halopéridol

ANNUAL REPORTRESEARCH GRANT

DA-93

INVESTIGATORJ. D. Spouge

University of British Columbia

During 1964 work on this project was complicated by the removal of the grantee from the University of Manitoba, to the new school of Dentistry in the University of British Columbia. Therefore, it was decided to complete as much work as it was possible to do in connection with certain minor projects and to write full reports on these (see enclosed MSS); to push ahead with a project which involved surveying clinical material from the local hospital

in the knowledge that this work could be extended and strengthened by a similar survey of material available in the Vancouver General Hospital; and to proceed with the work on Hypersensitivity Reactions in Mucous Membranes, with the knowledge that the new Department of Pathology with which I would be associated had a special interest in problems concerning hypersensitivity, and therefore facilities for special techniques were available subsequent to the move.

1. The effects of cavity preparation and the placement and polishing of gold-foil restorations in the teeth of dogs*

This project was completed and the manuscript submitted for publication. Shallow cavities were prepared by two different methods, and gold foil restorations were placed in the incisor teeth of dogs. Some of these preparations were polished, and some were not.

Results showed that high speed-spray cavity cutting appeared to produce less pulpal reaction than slow speed-dry preparation. Placement of gold foil produced marked pulpal disturbance, and gross damage resulted from the subsequent polishing of the restoration at the same sitting.

* Submitted for publication to the British Dental Journal.

2. A Comparative Review of Certain Locally Malignant Neoplasms of the Facial Region*

The basal cell carcinoma, the ameloblastoma, and the pleomorphic salivary adenoma exhibit marked clinical embryological and histopathological similarities. All the cases available at the teaching hospitals in Winnipeg were reviewed in order to analyse and accentuate any significance which may exist concerning these similarities.

The main object of the survey, however, was to use it as a means of acquainting ourselves with the practical problems involved, and the frontiers of modern knowledge concerning the subject. This was because we wished to utilise it as a starting-point for scientific histological investigation into the nature of the stem-cell of the pleomorphic salivary adenoma, which is now accepted to be of epithelial origin but whose cell of origin still remains clouded in doubt. It was hoped to utilise histochemical methods, polarised light microscopy; and fluorescent and electronmicroscopy in order to compare cellular detail, cellular degeneration products, and supporting ground-substance of both normal and pathological salivary tissue from humans, mammals, and fetal material in an effort to identify this stem-cell.

3. Hypersensitivity Reactions in Mucous Membranes.

This work was a continuation of previous experiments in this field. Our present investigations were aimed to ascertain the minimum dosage of certain recognised allergens which would produce clinical ulceration in the guinea-pig mucosa. Also we desired to ascertain if clinical ulceration could result with any degree of frequency by conditions simulating those found naturally in the mouth - i.e. surface application with penetration facilitated by exposure to trauma and excoriation.

Results showed that clinical ulceration could be produced by injection of very small amounts of allergen, but surface-diffusion produced equivocal results. It was hoped that improved facilities in our new location (U.B.C.) will enable to investigate penetration of allergen at the histological level, by the visual aid of fluorescent microscopy and 'tagged' antibodies.

4. Prognosis of Erupted teeth in the Newborn* - (Minor Project).

Pathology is the application of basic-science principles to diseased tissues, and hence scientific investigation into clinical material is justified. This project was therefore carried out as a minor exercise by a Summer Student-Assistant, between the periods when he was actively engaged in the more major projects described in this report.

The clinical problem involved an erupted tooth in a newborn child, which led to the histopathological study of the extracted tooth, and a review of the problem in general. We found that the literature contained suprisingly little scientific material on the subject. In the light of our histopathological findings it is probable that many of these natal teeth are not fully viable, and hence not deserving of the often strenuous attempts made to retain them.

The incidence is only 1 in 2-3,000 live births, and collection of a significant number would take a relatively long time. Therefore it was decided to approach the problem from a different aspect. The teeth involved are usually I/I and often become loose and are shed. Therefore we started to survey the kindergarten classes and early grades of schoolchildren to find any who had lower incisor teeth missing, and to check whether these fell into the appropriate category. Several cases were found, and the dental, medical and family histories were reviewed - and more especially the presence or absence of any resulting orthodontic problems.

It is hope to continue to occupy part-time of future Summer Student-Assistants with this scientific clinical survey.

* "Erupted teeth in the newborn", by J.D. Spouge and W.H. Feasby.

APPENDIX T

ANNUAL REPORT

RESEARCH GRANT: DA-128

INVESTIGATOR: J. R. Trott, University of Manitoba

TITLE: Gingival hyperplasia and gingival recession

- (A) 769 Winnipeg High School children were examined during last winter and the results recorded on the enclosed form. Initial breakdown shows that there were 384 males and 385 females in this group. The data has now been punched on I.B.M. cards and will be analysed this summer with the aid of funds from the Public Health Department.
- (B) A record for the reason for the extraction of teeth in 1688 patients was made on the enclosed form. This material has now been punched on I.B.M. cards and it is hoped to analyse this work this summer.

To complete the work on parts A and B, a request has been made to the Public Health Department as it was felt this may be a more suitable place to obtain funds for this type of research.

Two reasons why more has not been accomplished this year are first that I was a visiting research professor to the Graduate School at the University of Illinois from May 1st to June 31st., and secondly I have been charged with the responsibility of organizing and giving a new course in Oral Pathology at this University.

APPENDIX U
ANNUAL REPORT

RESEARCH GRANT

DA-94

INVESTIGATOR: J. Tuba
University of Alberta

TOPIC I. INFLUENCE OF INTESTINAL ABSORPTION ON BODY FLUIDS.

Part I. The Effect of Dietary Phosphorus on Human Saliva.

The ingestion of Na_2HPO_4 in gelatin capsules produced highly significant elevation of human salivary phosphorus levels. A simple meal containing about an equal amount of phosphorus had a similar effect. The consumption of the same meal followed at once by Na_2HPO_4 in gelatin capsules resulted in a still significant but modified effect on salivary P. This report is now based on 2 experiments, one year apart.

Part II. The Influence of Force-Fed Phosphates on Phosphorus and Alkaline Phosphatase in Rat Serum and Intestine.

Last year's report included evidence that force-feeding of Na_2HPO_4 to rats increased salivary phosphorus significantly. The transfer from intestine to saliva is mediated by the blood, in light of the present report. Both Na_2HPO_4 and sodium- β -glycerophosphate influence significantly the blood levels of Pi and calcium: phosphorus ratios. Serum alkaline phosphatase activity was unchanged by force-fed phosphates, CaCl_2 , CaCl_2 + lactose. Intestinal alkaline phosphatase activity was significantly increased by Na_2HPO_4 and sodium β -glycerophosphate. It was surprising to find that CaCl_2 feeding also resulted in enhanced activity of the intestinal enzyme; this was modified by simultaneous feeding of lactose. A few more experiments are now being done in an attempt to explain our findings. In the discussion, consideration is given to a number of factors known to be involved in intestinal absorption, and their possible relation to our results.

The evidence proves that ingested phosphate influences salivary phosphorus in humans and in the rat and that this effect is systemic.

TOPIC II. PROTEIN-BOUND CARBOHYDRATES IN GINGIVAL TISSUE.

Neuraminic acid content of gingival tissue in healthy and in diseased states was reported last year. We have done several normal dental papillae this year. The results are yet to be classified, but there is further evidence of significant variations. No written report is attached, although one will be available in February.

1. Binary Gold Alloys

The purpose of this research is to determine whether the properties of gold alloys, such as strength and corrosion, may be improved in whole or part, by other elements which retain the required mechanical and corrosion-resistant properties of conventional gold alloys. The gold-silver, gold-chromium, and gold-nickel binary alloy systems were investigated. The strength of the gold alloys increased substantially with addition of each of the alloying components. Gold-silver alloys showed the most pronounced increase, gold containing over 25 percent silver showing an increase of resistance to corrosion of a factor of 10. Gold-chromium alloys showed an increase in resistance to corrosion of a factor of 10. Gold-nickel alloys showed an increase in resistance to corrosion of a factor of 10. Gold-silver alloys showed an increase in resistance to corrosion of a factor of 10. Gold-chromium alloys showed an increase in resistance to corrosion of a factor of 10. Gold-nickel alloys showed an increase in resistance to corrosion of a factor of 10. Further work on the gold-silver system is warranted.

2. Gold-Porcelain Bonding

Cerium was added as an alloying component (up to 0.75%) to gold alloys to determine the effect on the strength of the gold alloy-porcelain bond. Cerium was a high vapor pressure in gold alloys, and thus its presence in gold alloys provides a variable component which would migrate into the porcelain. This should increase the diffusion rate between the gold and porcelain, thus resulting in faster and stronger bonding during firing operation. Preliminary results of this investigation show a definite increase in bond strength increase with additional cerium. However, the results were somewhat sporadic because of the difficulty of the testing technique. Further investigation is necessary to determine the effect of cerium on the bond strength of the gold-porcelain bond.

APPENDIX V
ANNUAL REPORT

W. V. Youdelis, University of Alberta

DENTAL MATERIALS RESEARCH - 1964

NCR DA-95

Three projects on research in dental materials were undertaken this past year: (1) an investigation of several binary gold alloys; (2) an investigation of the gold alloy-porcelain bond, and (3) an investigation of calcium fluoride in amalgam as a source of fluoride ions for caries prevention.

1. Binary Gold Alloys

The purpose of this research is to determine whether the precious metals in gold alloys, such as platinum and palladium, may be replaced, in whole or part, by other cheaper metals, while still retaining the required mechanical and corrosion-resistant properties of conventional gold alloys. The gold-cobalt, gold-chromium, and gold-tantalum binary alloy systems were investigated. The hardness of the gold alloys increased substantially with addition of each of the alloying components (1 to 5%). Gold-tantalum alloys showed the most promising results. Gold containing over 2% tantalum showed no shrinkage on solidification, and retained a pale white gold color up to 5% tantalum with good oxidation resistance. Gold-chromium alloys showed excessive solidification shrinkage and poor oxidation resistance. Gold-cobalt alloys showed more solidification shrinkage and oxidation tendency than gold-tantalum alloys but less than the gold-chromium alloys. Further work on the gold-tantalum system is warranted.

2. Gold-Porcelain Bonding

Germanium was added as an impurity constituent (up to 0.75%) to gold alloys to determine its effect on the strength of the gold alloy-porcelain bond. Germanium has a high vapor pressure in gold alloys, and thus its presence in gold should provide a volatile constituent which would tend to migrate out of the gold alloy into the porcelain. This should increase the diffusion rate between the gold and porcelain, thus resulting in faster and stronger bonding during the firing operation. Preliminary results of this investigation did show a noticeable trend of bond-strength increase with additions of germanium. However, the results were somewhat sporadic because of the difficulties inherent in the testing procedures. Further investigation is necessary to establish to what extent germanium increases the shear strength of the gold-porcelain bond.

W. V. Youdelis

3. CaF₂ in Amalgam

A study of the thermodynamics of the CaF₂ - H₂O system showed that CaF₂ may be used as a source of fluoride ions in amalgam for the prevention of caries (recurrent). Changes in the mechanical properties of the amalgam due to the addition of CaF₂ powder (up to 0.5% by weight) were found to be negligible. A paper on this investigation has been submitted for publication.

NOT FOR PUBLICATION

COPY NO. 24

NATIONAL RESEARCH COUNCIL OF CANADA

PROCEEDINGS
OF THE
SEVENTEENTH MEETING
OF THE
ASSOCIATE COMMITTEE ON
DENTAL RESEARCH

OTTAWA

22 OCTOBER 1965

NATIONAL RESEARCH COUNCIL

Associate Committee on Dental Research

EXECUTIVE

SEVENTEENTH MEETING

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1. Attendance:

Dr. J. E. Lusk, Chairman
 Dr. E. V. Blackman
 Dr. C. M. Jones
 Dr. E. H. Brubaker
 Dr. E. W. Cline
 Mrs. J. A. Van Dine

Dr. F. T. Jones, Vice-President (Administration)
 of the National Research Council, followed the Executive
 for the first part of the meeting. In the absence of
 Dr. E. W. Ballard, President, he delivered the opening
 remarks.

2. Chairman's letter to President of N.R.C.

On 18 September, Dr. J. E. Lusk had written
 to Dr. E. W. Ballard regarding:

a) that consideration be given to establishing
 special grants in the form of "Special Dental
 Grants" to be used to support post graduate
 students in dental research and to
 cover graduate students' expenses.

NATIONAL RESEARCH COUNCIL

Proceedings

of the

Seventeenth Meeting

of the

Executive

Associate Committee on Dental Research

22 October 1965

The Meeting convened in the Board Room of the National Research Council Administration Building, Ottawa, at 9:30 a.m., 22 October, 1965.

1. Attendance:

Dr. J.-P. Lussier, Chairman

Dr. R. V. Blackmore

Dr. C. M. Dowse

Dr. R. M. Grainger

Dr. S. Wah Leung

Mrs. J. A. Van Laer

Dr. F. T. Rosser, Vice-President, (Administration) of the National Research Council, joined the Executive for the first hour of the meeting. In the absence of Dr. B. G. Ballard, President, he delivered the opening remarks.

2. Chairman's Letter to President of N.R.C.

On 28 September, Dr. J.-P. Lussier had written to Dr. B. G. Ballard requesting:

- a) that consideration be given to establishing special grants to the deans of Canadian dental schools, to be used to support pilot studies, purchase smaller pieces of equipment, and to cover graduate students' expenses;

- b) that a small number of postdoctorate fellowships be allotted for support of dental candidates, in order to encourage dental research by creating opportunities for graduating or recently graduated Ph.D.'s..

3. The President's Reply

On 21 October, Dr. B. G. Ballard had replied to the Chairman's request.

With respect to deans' grants, he informed Dr. Lussier that any adjustment in Council policy to provide for these would be undesirable until action on recommendations proposed in the Report of the Bladen Commission was agreed upon.

In his letter, Dr. Ballard also drew attention to the discontinuation of the National Research Council's program of university postdoctorate fellowships, and noted that these were now to be included under the grants-in-aid program.

4. Opening Remarks: Dr. F. T. Rosser

Since Dr. Ballard was unable to be present to elaborate on his letter, Dr. F. T. Rosser attended the meeting in his place, and explained aspects of the Council's financial situation to the Executive.

Finance:

He drew attention to the science and engineering Grant Selection Committees who were competing for the annual appropriation from parliament, and emphasized that Council tried to be fair to all of them. To substantiate this point, Dr. Rosser referred to financial summaries indicating the amounts allotted to each Committee for grants-in-aid; the percentage of money awarded as compared with that requested in each case; and the number of grants awarded by the various Committees.

Deans' Grants: General Research Grants:

Dr. Rosser suggested that in place of grants to deans of dental schools, money be appropriated from the university presidents' general research grants for the support of dental research as desired by the respective schools. It was pointed out, however, that the policy in each university varied with respect to the use of the president's grant, and thus in some cases precluded the use of the money allotted as might be desired by certain faculties. After some discussion, it was agreed that this matter was beyond the control of the Council or the Associate Committee and would have to be worked out with the university authorities concerned.

"Priming" Grants

The Chairman then pointed out that the relatively new field of dental research was in a less advantageous position than other areas of science, and thus needed a substantial "priming" grant to boost it to a comparable level.

It seemed, he noted, that neither the universities nor government was willing to invest the additional amount necessary to fill the backlog, and enable dental faculties to secure personnel, equipment, etc., to meet accepted standards.

Although Dr. Rosser informed the Executive that in certain other weak areas of science such a "priming" grant had been awarded; he noted, however, that it had been mainly in support of an institution; and that grants to set up laboratories, of the type believed necessary in the case of dental research, were against Council policy.

Report of the Bladen Commission:

A brief discussion followed concerning the Report "Financing Higher Education in Canada", and the recommended increases for support of university research contained therein; this included a consideration of actual anticipated increases in N.R.C. grants for the coming year.

Postdoctorate Fellowships:

Dr. Rosser then briefly outlined the new program of postdoctorate fellowships administered under grants in aid of research, and drew attention to a few of the problems likely to be encountered in their administration.

A brief discussion on postdoctorate fellowships continued after Dr. Rosser's departure, during which the Executive expressed interest in information regarding the eligibility of dental candidates for postdoctorate overseas fellowships, or the possibility of creating such an award for Ph.D.'s in dental research. The Secretary was asked to obtain any available information.

5. Introduction of New Members of the Executive:

The Chairman welcomed Dr. R.V. Blackmore, Dr. C. M. Dowse, and Dr. S. Wah Leung as new members of the Executive, and introduced Dr. Dowse as a new member of the Associate Committee. He noted that the Executive now had a cross-Canada representation.

6. Executive Action Since March:

The Chairman drew attention to the following Executive Action taken since the Thirty-first Meeting of the Associate Committee on Dental Research:

1) Membership of the Committee:

Dr. G. E. Connell,
Dept. of Biochemistry
University of Toronto,
Toronto, Ontario;

was unable to accept appointment as member of the Associate Committee on Dental Research.

In his place, the Committee agreed to nominate:

Dr. Edouard Pagé
Dept. of Biology,
University of Montreal,
Montreal, Quebec.

At its June 1965 meeting, Council approved this appointment; Dr. Pagé accepted.

2) Fellowships:

A. Travel Grants

(i) Dr. D. G. Gardner, who held a Dental Fellowship from 1963-65, applied for a travel grant towards the cost of travel from Indiana University, Indianapolis, to the University of British Columbia, where he took up a position as Assistant Professor in the Department of Oral Medicine. Dr. Gardner was granted a travel allowance of \$257.66.

(ii) Dr. G. W. Thompson, who received a Fellowship for 1965-66, requested a travel grant towards the cost of travel from Edmonton to the University of Toronto, where his Fellowship is tenable. Dr. Thompson was granted a travel allowance of \$112.00.

B. New Applications:

Dr. L. D. Catena applied in July 1965 for a Dental Fellowship to be held at the University of Toronto. It was agreed that there were not sufficient reasons to make a strong case for this application. Accordingly, Dr. Catena was requested to apply again in March 1966.

C. Fellowship Declined

Dr. Clifford Young, awarded a Dental Fellowship for 1965-66 of \$3,500 to study at the University of Toronto, declined his award to begin practice as a dental surgeon in Pembroke, Ontario.

D. Request for Supply and Travel Grant

A request received from Dr. Paul Goldhaber, Director of Postdoctoral Studies, Harvard School of Dental Medicine, that N.R.C. Dental Fellows be provided with a \$500 supply and travel grant in addition to the normal stipend, was rejected by Council at its June meeting.

3) Grants

Operating Grant Awarded:

Lucas, O.N. The effect of barbiturates or tranquilizers combined with indirect anticoagulants on hemorrhagic manifestations and on prothrombic time on rats
Dentistry,
Alberta (Ed.) Requested: \$7,450
Awarded: \$4,000 DA-140

Operating Grant Reduced:

Marshall, F.J. DA-83: Autoradic graphic studies of dentine permeability.
Endodontol. &
Oral Histol., Original Grant: \$2,000.00
Manitoba Refunded: 303.72
Net Grant: 1,696.28

Accepted appointment at the University of Pittsburg.

Transfer of Grantee:

Castaldi, C.R. DA-121: 1964-65 The influence of water borne fluorides on human tooth morphology: \$2,200
Dentistry,
Alberta (Ed.)

Dr. Castaldi originally requested the transfer of \$200 to the University of Manitoba, where he moved. Later he decided to have this unspent balance at Alberta administered by co-worker Dr. W. Simpson.

Council Policy: Grants

The Secretary drew the Committee's attention to changes in Council policies outlined in the green "Special Notice", issued 1 October 1965 to all grantees, heads of departments, deans and university business officers; and requested that these be given close attention in the preparation of 1966-67 grant applications.

7. Business Arising Out of Executive Action:

A) Revision of "Regulations Governing Graduate Dental Research Fellowships"

The Secretary informed the Executive that travel allowances for N.R.C. scholarship and fellowship holders were now based on air economy, rather than railway coach fares. The Executive agreed that Dental Fellowships regulations be revised in accordance with this. The allowance for Dr. D.G. Gardner and Dr. Gordon Thompson had been decided by Council on the basis of air economy fares.

In the study of Dental Fellowships regulations, Dr. R.V. Blackmore noted that Items 1 and 4 were inconsistent, and recommended that Item 1 be revised as follows:

"The National Research Council offers a limited number of Graduate Dental Research Fellowships designed to provide advanced training and experience in one of the sciences basic to dentistry. Fellowships will not be awarded for the purpose of providing practical training and experience in the clinical branches of dentistry."

The Executive agreed to accept this revision.

B) Supply and Travel Grant: Supplement to Dental Fellowships:

The Executive discussed at length the policy of awarding a supply and travel grant with each Dental Research Fellowship. Since the request for this type of award had come from one source only, there was some disagreement as to the general necessity for implementation of the Fellowship by such means.

However, it was eventually agreed that, to attract more people to do research at this level, it would be desirable to supplement the Fellowship in some way.

It was therefore MOVED by Dr. R. M. Grainger, and SECONDED by Dr. R. V. Blackmore that a subcommittee be formed to consider means of providing further support for the Fellowship scheme, in the light of the request made by Dr. P. Goldhaber

CARRIED

The Executive agreed that this could be considered from the point of view of increasing the level of the award, and/or of providing a supply and travel grant.

8. Chairman's Report re: Estimates for 1966-67:

The Executive then turned its attention to a consideration of a letter written by the Chairman to the National Research Council, outlining estimated requirements for dental research during 1966-67. In summary, these are as follows:

Fellowships	\$78,200
Associateships	34,600
Scholarships	34,600
Grants-in-Aid	
Term	70,500
Annual-renewals	149,400
-new	56,000
Summer	
Assistantships	14,000
Equipment	60,000
Total	497,300

The Secretary reminded the Executive that these figures had been used by the Council in the preparation of the 1966-67 overall estimates, presented to parliament in September 1965.

Careful examination was made of the breakdown in each category, and it was agreed that the estimates had been modest.

Discussion followed with regard to the Associate Committee's policy in submitting and reviewing applications. There was general agreement that dental researchers were reticent about requesting large grants, and that this has been one reason why the Committee has never been able to provide adequate support.

9. Graduate Dental Research Fellowships: New Applications:

Muroff, F.S. To carry on research in histopathology, in the Dept. of Endodontics, Boston University, School of Graduate Dentistry, under Drs. H. M. Goldman and M.P. Ruben.

The Executive agreed that Dr. Muroff was not eligible for a Fellowship, since his work was basically clinical, and in a specialized field. The fact that the program at Boston is not research oriented was also taken into account.

Najjar, T.A. To study in the Dept. of Pathology, McGill University, under Dr. D.S.Kahn.

Since Dr. Najjar had not submitted the appropriate letters of recommendation, an academic record of his program at Yale, a clear outline of his proposed research, nor any information regarding the state of his advancement towards a doctorate degree; it was agreed that no decision could be reached without further information.

The Executive therefore decided to postpone decision until the Chairman had obtained necessary details from authorities at McGill University. If these revealed that a strong case could be made for an award to Dr. Najjar at this time, the Executive agreed to reconsider his request.

10. Report of the Subcommittee on Dental Fellowships:

The Executive considered at length the report presented by Dr. R. M. Grainger, Chairman of the subcommittee formed in March to study the problem of dental fellowships.

In conclusion, the members agreed that, since the policy of the Associate Committee on Dental Research is to support graduate research, but not training for clinical specialities; therefore individuals requesting support should be required to submit, with their application, an

outline of the studies to be undertaken in the year, or years for which support is requested.

Thus an individual taking a Master of Science Course in Bacteriology or Histopathology, and wishing to undertake research in periodontal disease, could expect support for all years. However, a candidate in a course leading to a certificate in the specialty, followed by training in basic science, could expect support only for the latter years in basic research training. Finally, those in a course offering both specialist and research training, the two types taught at the same time, would be eligible for fellowships proportional to the amount of research training incorporated in the whole course as if it had been undertaken in a separate year or years.

The Executive agreed, however, that these changes would not affect those already pursuing their studies under the dental fellowship program.

It was also recommended that applications from candidates in specialist training be directed to the Department of National Health and Welfare. However, problems which could arise in this respect were pointed out; and consequently, after some discussion, it was decided to contact the Department of National Health and Welfare to find out what their reaction to this plan would be.

11. Report of the Committee on the Statement Relevant to
Dental Research and the Health Sciences Research
Council

The Executive considered the first draft of this report, commenting and suggesting certain changes. Following a brief discussion, the members agreed to leave it in the hands of the Committee to prepare further changes, in order that a final version could be considered by the Associate Committee in March 1966.

12. Membership of the Committee;

The Chairman noted that the appointment of a

replacement for Dr. G. Nikiforuk had been overlooked in March. It was suggested that a representative from the University of Western Ontario be nominated, since the new dental school had just opened there.

It was therefore MOVED by Dr. R. M. Grainger, and SECONDED by Dr. C. M. Dowse, that Dr. W. Dunn, Dean of the Dental School of the University of Western Ontario, be asked to suggest the names of those in the basic sciences at that university who might be willing to serve as members of the Associate Committee on Dental Research.

CARRIED

13. Unspent Balances of N.R.C. Grants:

The Secretary drew attention to the large numbers of N.R.C. grantees who were not spending their present grants, leaving substantial unexplained balances every year. It was noted that a number of dental grantees fell into this category; and therefore, the Executive recommended that the guilty parties be informed by letter and requested to supply an explanation.

14. Letter from Dr. W.V. Youdelis:

Brief attention was given to a letter from Dr. W. V. Youdelis, justifying his position in the light of the severely critical reviewers' comments on his 1965-1966 application. The Chairman had previously replied to this letter. It was agreed, therefore, that no further action needed to be taken; and that Dr. Youdelis' comments would be taken into consideration with respect to future applications received from him.

15. Revision of Schedule: Meeting of the Associate Committee on Dental Research and Executive - March:

A recommendation was presented to the Committee, proposing that the March meeting of the main Committee take place prior to the meeting of the Executive, a reversal from past procedure.

Assessment of Applications

a) Evaluation of Applications:

The members of the main Committee would evaluate each application and recommend an amount deemed appropriate, without reference to the total amount available; which they would not be told. These evaluations would be recorded and sorted by the Executive at their meeting on the day immediately following.

According to the available funds, the Executive would be responsible for "funding down" each recommendation as necessary, and distributing the money appropriately.

With respect to the discussion of applications, it was also recommended that each member leave the room as the applications submitted from his university were being considered.

b) Estimates

It was recommended as well that the estimates for the following year be drafted by the Executive at its meeting in March. These would then be immediately available when requested by Council in September.

16. Date of the Next Meeting:

The Executive requested the Secretary to try to arrange for the annual spring meeting to be held in late February or early March, and to reserve three days for the meeting of the main Committee and one for that of the Executive.

The meeting adjourned at 4:45 p.m.

Distribution List

(i) to members of the Associate Committee on
Dental Research

- | | |
|------------------------|------------------------|
| 1. Dr. J.-P. Lussier, | 11. Dr. D. P. Hill |
| 2. Brig. K. M. Baird | 12. Dr. A. M. Hunt |
| 3. Dr. B. G. Ballard | 13. Dr. C. P. Leblond |
| 4. Dr. G. H. Beaton | 14. Dr. S. Wah Leung |
| 5. Dr. R. B. Blackmore | 15. Dr. E. Pagé |
| 6. Dr. G. M. Brown | 16. Dr. G. J. Parfitt |
| 7. Dr. R. A. Connor | 17. Dr. A. J. Rhodes |
| 8. Dr. C. M. Dowse | 18. Dr. D. M. Tanner |
| 9. Dr. F. W. Fyfe | 19. Mrs. J.A. Van Laer |
| 10. Dr. R. M. Grainger | |

(ii) to other persons

20-24 Deans of Dental Schools (who are not members of
of the Committee)

- | | |
|---------------|-------------------|
| 20. Alberta | Dr. H. R. MacLean |
| 21. Dalhousie | Dr. J. D. McLean |
| 22. Manitoba | Dr. J. W. Neilson |
| 23. McGill | Dr. J. McCutcheon |
| 24. Toronto | Dr. R. G. Ellis |

25. Dr. F. T. Rosser

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